URINARY INFECTION AND ORAL PENICILLIN G

by

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"It can be counted fortunate in this respect that the supplies of penicillin at the earliest stage of clinical application were so meagre, and the material so valuable, that a considerable degree of control could be maintained and a high standard of clinical work enforced. As a result its mode of use was firmly based on facts which had been ascertained by experiment in the laboratory, thus ensuring within the limits of experience at the time the best use of the material in almost every case".

Florey, et al. 1949.

"Had penicillin G been given originally in doses of 1 - 2 g per day, like tetracycline and chloramphenicol, it might have been classified as a broad spectrum antibiotic".

Stewart, 1965.

TABLE OF CONTENTS

SECTION III

LIST OF FIGURES

ACKNOWLEDGEMENTS

A cursory glance through the thesis which follows will reveal that the author is indebted to many people without whose help the work would not have been possible, and there are others whose contribution is not immediately obvious. To Professor Macdonald I am particularly grateful because it was he who first drew my attention to Vinnicombe's (1966) report on the treatment of patients with penicillin, and suggested that this unusual claim ought to be investigated. His interest and suggestions, and those of the members of the staff of the bacteriology department in Aberdeen University were a constant encouragement.

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Without the assistance of the general practitioners the clinical trial could not have been carried out. Their names appear overleaf.

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DECLARATION

I declare that this thesis records the results of experiments carried out by me, that it is my own composition, and that it has not been previously presented for a higher degree.

March 1st, 1971.

SUMMARY

1. A review of the literature on the absorption of oral penicillin and its excretion, the relative importance of serum and urine levels of antibiotics in the treatment of pyelonephritis, and on the penicillin sensitivity of Gram-negative urinary pathogens is given, and it includes a comment on the published cases of Gram-negative urinary infection treated with oral penicillin.

There are also assessments of the laboratory diagnosis of urinary infection in patients consulting general practitioners, and of the importance of this disease in general practice. Penicillin resistance is discussed with particular reference to the development of penicillinase-producing resistant mutants, and an account of some recent experiments with an in-vitro model bladder is given.

2. Studies with six volunteers demonstrated that the oral administration of a single dose of 500 mg of penicillin G produced mean urine levels of 500 µg of penicillin per ml in the first 2 hours, and that in the urine secreted between the 4th and 6th hours after the dose the mean concentrations were 40 µg per ml. Serum concentrations were approximately 1000 times less. Three different preparations of oral penicillin were examined in a cross over trial, and the cheapest, which was "Crystapen G" (potassium penicillin G) was found to be the best.

3. The penicillin sensitivity of 969 strains was measured. These included 502 consecutive strains isolated from patients with urinary infections who were attending their general practitioners, 48 strains from antenatal patients with bacteriuria, 243 strains from patients with urinary infections in hospital, and 176 strains from the faeces of healthy adults. There were in all 608 strains of Escherichia coli, 106 strains of Proteus, 12 of Ps. aeruginosa and 35 strains of Gram-positive cocci. Eighty three per cent. were sensitive either to a specially

developed filter paper disk, containing 100 µg of penicillin, in a disk-diffusion test, or to 50 µg per ml in a tube-dilution test. The results of these two tests were found to be equivalent.

4. All the penicillin-resistant strains produced free penicillinase, whereas none of the penicillin-sensitive strains did so under normal conditions. A sensitive strain was found to produce small quantities of bound penicillinase under normal conditions, and small amounts of free penicillinase when incubated for seven days. Sensitive strains grown in sub-lethal concentrations of penicillin were found to become penicillin-resistant, and two out of three such strains became free penicillinase producers. The mean inhibitory concentration of one of these strains increased from 25 to 5000 µg of penicillin per ml. The changes in penicillin sensitivity were stable on culture in penicillin-free media and on storage.

5. A computer was used to test the relationship between penicillin resistance and ampicillin resistance, and also to investigate possible links between the resistance of other pairs of antibiotics. There was among the urinary strains of E. coli evidence of an association of resistance between streptomycin and sulphonamide and between streptomycin and tetracycline, and to a lesser extent between tetracycline and sulphonamide.

6. An in-vitro model bladder was set up and the rapid elimination of penicillin sensitive Gram-negative urinary pathogens was observed when they were exposed to concentrations of penicillin similar to those shown to occur in the volunteers and patients. A strain of E. coli resistant to the concentrations of penicillin achieved in the bladder was also shown to suffer bacteriolysis. When the residual infected volume is being diluted the bacilli appear to be peculiarly sensitive to penicillin.

7. The method of testing the urine of patients attending general practitioners was examined. The dip-inoculum method was exhaustively tested in the laboratory and clinically. The difference in the relationship between the viable count as performed by a surface counting technique and the spoon count for strains of Gram-negative bacilli and Staph. aureus was shown to be due to clumping of the staphylococci.

8. The inoculation of the spoon by passing it through the uninterrupted stream of urine during micturition was tested, and though marginally less accurate in theory, it did not prove to be so in practice, and the method was considered to have considerable advantages. These were the ease with which the mid-stream specimen of even a small stream of urine could be sampled, the absence of discomfort or difficulty for the patient, and the lack of a need for a container in which to collect the urine.

9. The use of oral penicillin G was tested in four patients who developed bacteriuria while recovering from gynecological operations. The immediate response to treatment was encouraging, but unfortunately these patients could not be followed up adequately.

10. A single patient with acute urinary infection caused by a pure strain of Escherichia coli sensitive to 25 μg of penicillin per ml was given a single dose of 500 mg of penicillin by mouth, and her clinical and bacteriological response was followed for two days. The viable count of bacilli per ml of urine was reduced from 2.72×10^7 to 32 bacilli per ml over 5 hours, but after 12 hours it had returned almost to pre-treatment levels. The clinical improvement was considerable and maintained for nearly two days and then relapsed quickly. A course of penicillin G 500 mg 6 hourly by mouth was started and continued for

four weeks. The clinical and bacteriological response, though slower than after the first dose was nevertheless steady and complete. No alteration in the sensitivity of the infecting organism to penicillin was noted.

11. A second patient, a child, with an acute Gram-negative urinary infection was treated with a five day course of oral penicillin G, 500 mg per day in divided doses. Clinical and bacteriological improvement was dramatic and complete

12. A clinical trial of the use of oral penicillin G was established with the aid of 15 general practitioners. Five hundred and thirty patients were involved, of whom 242 were shown to be infected and were treated and followed up. Ninety four were treated with penicillin and 57 with sulphonamides, thirty of the former and 29 of the latter being treated 'double-blind' with specially formulated tablets. The remaining 91 patients were treated with other drugs. Follow-up analyses of the urine were performed in the third and seventh week after diagnosis. Therapy with penicillin was compared with sulphonamide and was found to be marginally better when the results were compared immediately after treatment stopped, and marginally worse after 7 weeks. The differences were not significant. The results were also compared with the smaller number of cases treated with other drugs, andwere analysed according to age and a history of previous infection.

13. Clinical and bacteriological cures were compared, and it was found that there was no correlation between the subjective opinion that treatment had been successful and the bacteriological record of elimination of bacteriuria. Continuing symptoms were however, a reliable indication of the failure of treatment.

14. The general conclusion is that penicillin is the cheapest, and in many ways the best 'broad-spectrum' antibiotic for the treatment of Gram-negative urinary tract infection.

INTRODUCTION

PREVALENCE AND IMPORTANCE OF URINARY TRACT INFECTION

Historical account

The urine, being the most readily available of the body's fluids has been most carefully observed and studied since time immemorial. Six thousand years ago Babylonian physicians made observations on its colour and consistency and Hippocrates (460 - 377BC) wrote an account of the diseases of the urinary tract in his Prognostics and Aphorisms. Progress in the understanding of urinary diseases was, however, slow, and the principal content of a 15th century Byzantine manuscript "On Urine" was still the description of the colour, texture and significance of 21 different specimens of urine.

In 1675 Loeuwenhoek described animalcules which he observed with his microscope, and which he thought came from the air where they existed as seeds. Others considered that the animalcules had arisen spontaneously by the combined action of heat, water, air and 'putrefaction'. It was not until 1858 that van den Broek demonstrated that normal urine taken 'aseptically' from the bladder would not ferment. His method was to kill an animal and then rapidly cut it open, tie off the urethra and ureters, remove the bladder which was then immersed in a bath of mercury. He cut open the bladder with a sterile knife and allowed the urine to ascend and fill an inverted vessel over the mercury. Some years later, in 1866 Pasteur also obtained sterile urine, and he noted that urine was a useful culture medium and grew anthrax bacilli in it (Bulloch, 1938).

By 1881, however, considerable strides had been made in the study of urinary tract infection. In that year William Roberts, M.D., F.R.S., Physician to the Manchester Royal Infirmary, read a paper entitled "On the occurrence of microorganisms in fresh urine" to the North Wales branch of the

British Medical Association. In the pre-amble he notes that "The fresh and healthy urine is perfectly free from bacteria or other minute organisms. The ordinary types of morbid urine, although they may contain blood, pus or casts of tubes, are equally free from organisms". He goes on to enumerate different types of urinary infection and describes what we would now call asymptomatic bacteriuria, and acute and chronic urinary infection. He describes urines containing bacilli and "Micrococcus chains". He knew that infection was commoner in women than in men, and he attributes this to the anatomy of the urethra, and he postulates an ascending route for most infections. He mentions many of the factors predisposing to urinary tract infection, even noting the importance of residual urine, and he was aware of the dangers of instrumentation; "A dirty catheter is a most efficient infective agent" (Roberts, 1881).

It took the medical world 75 years to become receptive to Robert's ideas on urinary tract infection, and so it was Kass who in 1956 again drew attention to the danger of catheterisation, to the need for quantitative culture of the urine, and to the importance of asymptomatic infection. Since this reawakening of interest, however, experiments and investigations, surveys and trials, embracing every aspect of the subject have resulted in such a volume of literature that a modern urologist can regard with wry amusement the comment of Cabot and Crabtree in 1916: "The literature on the subject is stupefying both in quantity and complexity, and anyone who has attempted to master it will, we think, be convinced of the fact that it is more likely to confound than enlighten the reader".

Urinary tract infection in the community

Of the general importance of urinary infection there can be no doubt. In

studies of the prevalence of bacteriuria in adults in communities as far apart as South Wales and Jamaica the overall prevalence was 0.5 per cent. of men and 4.4 per cent. of women. There were some differences in the prevalence among the women of the different communities. In rural Jamaica 4.9 per cent of the adult female population suffered from urinary infection while in rural Wales the rate was 6.6 per cent. In urban Jamaica the prevalence was only 2.2 per cent., whereas in the mining villages of Wales it was 4.9 per cent. The prevalence also changed notably with age increasing steadily, with no peaks, from 2 per cent. for the 15 - 24 year olds to 5 per cent. for the 45 - 54 year group and then steeply up to 16 per cent. for the over 65's, (Kass, Savage and Santamarina, 1965). The lower rates in Jamaica may be related to race for Kunin and Paquin (1965) noted that negro children suffer from fewer urinary infections than their caucasian peers, and also that relapses and re-infections following treatment are more rare among these children. Spontaneous remissions occur in about a quarter of the bacteriuric population every year, and they are replaced by an equal number of new cases or relapses (Asscher, 1970).

Progress of the disease

That pyelonephritis is a dangerous condition is well proved. In the short term, acute pyelonephritis causes much pain and discomfort, and may go on to septicaemia and death. Occult bacteriuria is associated with renal disease in children (Savage et al., 1969), although the nature of this association awaits further study. In pregnant women bacteriuria pre-disposes to acute urinary infection (MacDonald et al., 1957) and probably to pre-eclamptic toxaemia, prematurity and foetal loss (Kass, 1959; Stuart, Cummins and Chin, 1965; Gruneberg, Leigh and Brumfitt, 1969), and this tendency can

be reversed with treatment of the bacteriuria.

In the medium and long term pyelonephritis is associated with retarded growth in children (Savage et al. 1969), and in adults with hypertension and therefore with the morbidity and mortality that this brings. Since hypertension is familial and bacteriuria is not it is likely that bacteriuria is causally related with only a proportion of hypertension (perhaps as much as 20 per cent.) and it may be that it accounts to a considerable extent for the excess proportion of hypertension found in elderly females compared with males (Kass, Savage and Santamarina, 1965). In the long term the damage from pyelonephritis may be so severe that there is insufficient healthy renal tissue left to carry out the essential excretory functions of the body with resulting uraemia, and death.

Post-mortem studies

Much pyelonephritis goes undiagnosed until death. Kimmelsteil and his colleagues (1961) review briefly the incidence of pyelonephritis diagnosed at necropsy from 10 published sources, and found that it varied from 1.9 per cent. to 20 per cent. with most authors giving a figure near 5 per cent. With strict pathological criteria Kimmelsteil's own figure was 2.8 per cent. of 3,939 routine necropsies, with less strict criteria his figure rose to 5.8 per cent., and he notes "Chronic pyelonephritis can be recognised much more often at autopsy than during life".

In the United States of America (Kessner and Florey, 1967) and in the United Kingdom (Waters, 1968) studies have been made in the mortality trends from urinary tract infections, and it seems possible that there is an absolute increase in the actual mortality from this condition, although no cause for this phenomenon has been proposed.

Urinary infection in different population groups

Certain sections of the population are more at risk than others. With the exception of neonatal boys, the female sex is more prone to urinary infection than the male at all stages of life. The neonatal excess of males is probably due to the higher incidence of malformation of the urogenital tract in males.

Some population groups have been investigated for urinary tract infection more thoroughly than others. Perhaps more effort has been expended in studying urinary infection and its consequences in antenatal women than in studying all other groups together, due no doubt to the fact that this 'captive' group attends regularly at clinics, is generally youthful, reasonably active and co-operative. As long ago as 1931 Dodds showed that 7.6 per cent. of antenatal women developed persistent bacteriuria, and in 1960 Kass demonstrated that unless treated 40 per cent. of them developed acute pyelonephritis later in pregnancy or in the puerperium.

The section of the population in which the prevalence and importance of urinary tract infection is now being evaluated is the school entry group. Kunin and his colleagues (Kunin, et al., 1960, 1962, 1965) have examined 16,000 school girls and have found a prevalence of urinary infection of 1.2 per cent. compared with 0.03 per cent. in boys. Two surveys in the United Kingdom, one in Dundee (Savage et al., 1969) and one in Birmingham (Meadow, White and Johnston, 1969) confirm these findings. Savage found that the girls with bacteriuria were smaller and lighter than their peers, and 70 per cent. of them had serious underlying renal disorders. More work is planned to determine the relation between the bacteriuria and the renal disorder, but Hodson claimed in 1965 that the kidneys of children with unarrested urinary infection

failed to grow, whereas with treatment growth was restored.

The emphasis may well shift to even younger children. Stansfeld (1966) considers that infection in children begins in infancy, and especially in the first month, and Smallpiece (1966) in his review of the aetiology of urinary infection postulated that one reason for the severity (in terms of the incidence of progressive disease) was the fact that the resultant scar following a urinary infection in a child was large in relation to the size of the kidney. Vesico-ureteric reflux frequently accompanies urinary infection in young children, but whether it is the cause of consequence of urinary infection is unclear. The subject, moreover, is complicated by the surgical concept of bladder neck obstruction which may not be distinct from reflux (Smallpiece, 1966). McGregor et al. (1966) note that nocturnal voiding and recumbency are probably important factors in the aetiology of the disease. If a child voids when asleep any reflux which exists will exert more force on the kidney, and urine will not drain from the kidneys so readily. Learning to walk and gaining nocturnal control may therefore be important urological milestones.

Among the elderly the incidence of urinary infection in both sexes rises above that of any other age group. With males this is associated primarily with disease of the prostate gland, and with females with multiparity and gynaecological conditions. This group may contain many of the more intractable urinary infections (Jackson, Kozij and Jao, 1965), but it appears to have had least attention.

Reason for the preponderance of females with urinary infection

Stamey and his colleagues (1965) considered that there were four factors which accounted for the fact that women with urinary infection outnumber men by about 8 to 1.

These are:

1. The short length of the urethra.

2. The external 1/3rd of the urethra is continuously contaminated with rectal and vaginal flora.

3. It is probable that usually women do not empty their bladders as completely as do men.

4. During intercourse in a proportion of females the urethral orifice may become intravaginal.

Prevalence of E. coli in urinary tract infection

A single strain of Escherischia coli in a pure culture is the commonest finding in acute urinary tract infection. The actual proportion of infections caused by E. coli varies greatly from series to series depending on the population studied and on other factors. However, around 60 - 90 per cent. of acute uncomplicated infections are caused by E. coli whereas in chronic infections other organisms such as Proteus, Pseudomonas aeruginosa and Streptococcus faecalis are found more commonly and E. coli may be present in a pure culture in less than one in five cases. A pure strain of bacteria may be expected in 80 - 100 per cent. of acute infections whereas in chronic recurrent infections only 20 per cent. may be caused by a single strain of bacteria (Coleman and Taylor, 1949; Kass, 1955; Steensberg et al., 1969). In Coleman and Taylor's series of 60 patients with uncomplicated infections of the urinary tract and 40 patients with chronic infections or infections associated with some abnormality of the urinary tract the figures were 82 and 18 per cent. respectively.

In asymptomatic infection of the pregnant or non-pregnant woman the proportion of infecting organisms that are E. coli tends to be higher and

less variable: thus Sussman et al. (1969) found that 90 per cent. of such infections were due to E. coli, Little's figure (1966) was 90.5 per cent., and Turner's (1961) 87.9 per cent.

Serology of strains of E. coli

In the first half of the last decade a considerable amount of attention was paid to the serology of strains of E. coli causing urinary tract infections. Lowell Rantz writing on urinary tract infections in 1965 remarks "Most investigations of the parasite (E. coli) during the last few years have centered around further identification of E. coli by serologic methods".

Following the discovery that certain serotypes of E. coli were enteropathogenic causing gastroenteritis in infants a search for nephropathogenic strains of E. coli was instituted. About 12 serotypes, but especially serotypes 01, 02, 04, 06 and 075 were thought to be particularly liable to cause urinary infection (Rantz, 1962; McGeachie, 1965; Gruneberg, 1968), but Turck, Petersdorf and Fournier (1962) found that these serotypes were also more commonly isolated from the faeces of patients without urinary tract infections, and Spencer et al. (1969) found that in 80 per cent. of cases the organisms isolated from the urine were also present in the faeces. In McGeachie's series the 'nephropathogenic strains' formed the same proportion of strains from acute uncomplicated cases as from chronic infections, nor was there any difference in the proportion of strains which were 'nephropathogenic' isolated from cases with bacterial counts in excess of 100,000 organisms per ml compared with those with counts of less than 10,000, the majority of which were, presumably, contaminants. The meticulous investigation by Gruneberg (1969) confirms that a patient is most likely to acquire a urinary infection from the strain of E. coli commonest in her bowel.

This does not completely disprove the presence of nephropathogenic strains although it does rule out the possibility that a minority strain in the faecal flora by virtue of its increased virulence, can attack the urinary tract. Kennedy, Plorde and Petersdorf (1965) showed that certain serotypes associated with urinary tract infection were concentrated in the hospital environment, and that these strains were more able to persist in the gut and colonise the urethra than 'outside' strains. Prat et al. (1965) demonstrated that the pathogenicity of various strains of E. coli for the intact rabbit kidney could be increased by repeated passage on renal tissue. Of the serotypes which he tested strain 02 increased its pathogenicity most dramatically - from causing infection in 11 per cent. to 60 per cent. of in-vivo experiments. They noted incidentally, that the primary site of localisation of renal infection was in many cases the wall of the pelvis. Finally Jackson and his colleagues (1965) showed that nephropathogenic serotypes of E. coli occurred most frequently in older people with long standing infection.

Treatment of urinary tract infection

The treatment of urinary tract infection has undergone considerable changes since Roberts advised the members of the North Wales branch of the British Medical Association that the rational treatment for bacteriuria was to dislodge the 'colony' (the word is used with a 'Foreign Office' connotation) which had become established in the bladder with a solution of boracic acid. Garrod (1958) reviewed antibiotic-and chemo-therapy of urinary tract infection beginning with the effect of acidification of the urine and then dealing with the early synthetic drugs such as hexamine and mandelic acid. He discusses the sulphonamides and nitrofurantoin and reviews the use of tetracycline and

streptomycin and cycloserine. Since then (1958) the most notable developments so far as urinary infection is concerned have been the manufacture of the penicillin-related antibiotics, ampicillin, carbenicillin, and cephaloridine, the streptomycin-related antibiotics, kanamycin and gentamycin, and, most recently, trimethoprim and its use in combination with the sulphonamides. The extent of the choice of antibacterial agent can be guaged by noting that the cost of a 2 -week course of sulphonamide treatment is less than £1, while a similar course of carbenicillin at low dosage costs about £70.

Although a wide range of antibiotics are now available for the treatment of urinary infection, McGeachie (1966) does not consider that the recurrence rate is much lower than it was earlier. Further, in spite of constant propaganda about drug-resistant organisms, there has not been a large increase in the number of organisms resistant to many drugs ab initio. Indeed the proportion of strains of some species of bacteria that are resistant to tetracycline appears to be decreasing (O'Brien, Kent and Medeiros, 1969). It is clear that the majority of organisms, especially but not only in general practice, are sensitive to most if not all of the readily available drugs. Eykn and Phillips (1969) remark, in referring to urinary infection in the community, that "the responsible organism is usually a fully sensitive E. coli", and they go on to write "Of all the agents available a sulphonamide is still the first choice since it is effective, relatively non-toxic and cheap".

Sulphonamides are used extensively in general practice (Mond et al., 1965) and, where laboratory sensitivity tests are favourable, they are still used in hospital practice, especially for long term suppressive therapy (Normand and Smellie, 1965). Recently, following the discovery of

trimethoprim, and the demonstration that synergy between it and sulphonamide occurs, a combination of these two drugs is being used increasingly. However sulphonamide therapy remains the standard against which the efficiency of other first line urinary antibiotics and chemotherapeutic substances should be measured.

OUTLINE OF THESIS

The core of this investigation was stimulated by the work of T.A. Stamey and his colleagues (Stamey, Govan and Palmer, 1965). They calculated that following oral administration of penicillin in doses normally given for infections with Gram-positive organisms enough penicillin would be excreted in the urine to inhibit Gram-negative bacilli. They considered that it is sufficient for urine concentrations of the antibiotic used to exceed the minimum inhibitory concentration of the infecting organism for therapy to be effective and they demonstrated the successful treatment of ten out of twelve patients with proven pyelonephritis by oral administration of penicillin G or penicillin V. That such a cheap, non-toxic, and readily available drug, that is usually considered to have a 'narrow spectrum', might be suitable for use in this large field of practice demanded further attention.

The work falls naturally into three parts. In the first place the relation between the levels of penicillin G which could be achieved in the urine after oral therapy and the penicillin sensitivity of a large number of urinary pathogens had to be studied in some detail. In this section also the importance of penicillinase production is investigated and correlations are made between the penicillin resistance of a urinary pathogen and the species of the pathogen, its biochemical nature, its source and resistance to other antibiotics, and other factors such as a history of recurrent infection. A filterpaper disk with the appropriate amount of penicillin in it was developed for rapid sensitivity testing, and finally a model bladder was set up to test the hypothesis in a situation simulating as closely as possible that existing in vivo.

In the second section a method is developed for culturing the urines of patients living at a distance from the laboratory using the dip-inoculum spoons

of Mackey and Sandys (1965). In the first part of the final section a report on three episodes of proven urinary tract infection treated with penicillin G, and followed up very carefully, with many samples of urine tested over several months, is given in some detail. This is followed by a report of a clinical trial comparing penicillin with sulphonamide and other drugs (part of it double blind). Five hundred and thirty patients were involved from six general practices.

A computer was used to evaluate some of the bacteriological and clinical results. The purpose of this exercise was twofold. Firstly because of the increasing use of computers in clinical and laboratory medicine the author attended a course on computing, to learn a computer language and to learn how to write a computer program. To gain experience the analysis of some of the data for this thesis was carried out by the computer. Secondly, with a large number of isolates (nearly 1000) from different sources, most of them tested for sensitivity against 8 or 9 antibiotics; and with over 500 patients in the clinical trial, there was clearly a case for the handling of the information by a machine.

Before the report on the investigations carried out, the pertinant literature is discussed, and after the final section the main topics are discussed and summarised.

LITERATURE REVIEW

URINARY TRACT INFECTION IN GENERAL PRACTICE

Until recently most of what was known of urinary tract infection and pyelonephritis was gained from the study of patients in hospitals or attending clinics, and from post-mortem examinations. In the last decade, however, there have been important papers from practitioners in the United Kingdom (Fry et al., 1962; Loudon and Greenhalgh, 1962; Mond et al., 1965 and 1970; and Waters et al., 1970), and from New Zealand (Gallagher, Montgomerie and North, 1965) and Denmark (Steensberg et al., 1970) outlining the presentation, diagnosis and treatment of urinary tract infection in general practice, and describing its epidemiology and symptomatology. Furthermore, two statistical surveys have been published, one from the United Kingdom by the College of General Practitioners (Logan and Cushion, 1958), and one from Denmark (The Danish National Morbidity Survey, 1960) which provide a general picture of urinary disease in the community and of the work load that this imposes on the health services.

Between one and two per cent. of all consultations in general practice are for urinary tract infection, and yet this may represent only half the problem for many patients with symptoms of urinary infection do not seek medical advice about it. Of those who do consult their general practitioner only about half actually have bacteriuria, although a proportion of those that have sterile urine at the time of consultation may develop bacteriuria if carefully followed up. Kass, Savage and Santamarina (1965) using the figures from Jamaica and Wales quoted in the Introduction (page 3) estimated that between 10 and 20 per cent. of the total female population will develop urinary tract infection at some time in their life, but Waters in his community survey in the Welsh valley (Waters et al., 1970) found that 22 per

cent. of women had experienced dysuria in the past year and half remembered having dysuria at some time in their lives.

Age and sex distribution, and marital status

The ratio of men to women varied considerably in the reports mentioned above, from 1 in 12 (Gallagher et al., 1965) to 1 in 3 (Steensberg et al., 1969). In all the reports most patients were married women, but Steensberg and his colleagues showed that the prevalence per 1000 females per year in Copenhagen was highest (36 per 1000) among divorced women, and lowest (15 per 1000) among unmarried women.

Among the children in Mond's practice in London the annual incidence of urinary tract infection was 1.4 per cent., and of the 10 children found to have the disease 8 were infected before school age (Mond et al., 1970). Savage et al. (1969), who found an incidence of bacteriuria of 2.1 per cent. in 5-year-old school girls, noted that 3 of the 20 girls already had established pyelonephritis.

Gallagher, Montgomerie and North (1965) showed that the age distribution of patients consulting with symptoms of urinary infection showed a peak in the 16 - 25 age group with a fairly constant number of patients consulting in each of the older age groups. Steensberg and his associates confirmed this and showed in addition that the 20 - 40, and over 60 age groups had the highest number of patients per 1000 females per year, and he also demonstrated a steady increase in the rate of chronic urinary tract infection as age increased.

The 'urethral syndrome'

Mond and his colleagues (1965) and Gallagher and his colleagues (1965) have compared their clinical findings in patients presenting with symptoms

referring to the urinary tract who have bacteriuria with those who do not. The age distribution and the symptomatology and clinical history were almost identical in the two groups except that there were fewer cases with haematuria and pyrexia in the non-infected group, and the proportion of patients with symptoms but no bacteriuria declined in the over 60 age group. It was noted, however, that the diagnosis was made with more certainty in those who were later shown to have bacteriuria compared with those who had a sterile urine. It has been suggested that patients with symptoms but without bacteriuria are suffering from an allergy (Kindall and Nickels, 1949), 'non-specific infection' (Eberhart, 1958), congestion of the urethra (Ormond, 1935), obstruction (Davis, 1956), anxiety neurosis (Gray and Pingleton, 1956) and chronically infected urethral glands (Winsbury-White, 1960). Mond showed that 47 per cent. of these patients had significant pyuria which was not associated with vaginitis. Gallagher found that 28 per cent. of them developed bacteriuria if followed up for 3 months and was convinced that they suffered from the 'urethral syndrome' which he considered to be infection of the urethra and surrounding glands. However Murdoch et al., (1968) in their account of over 3000 patients presenting with urinary symptoms found that a third showed no abnormalities of the urinary tract, and did not develop bacteriuria even if carefully followed up. It may be therefore that the 'urethral syndrome' covers more than one clinical condition.

Importance of pyuria

The examination of the urine for the presence of pus cells is frequently carried out in hospital and general practice as an aid to the diagnosis of urinary tract infection. Where this is done in addition to quantitative or semi-quantitative urine culture it may add useful information, but where it

is the sole procedure it is totally inadequate. Both false positives and false negatives will occur.

Following operations on the urogenital tract, in the presence of inflammation of the female uro-genital tract, or of balanitis or urethritis in the male, pyuria may be present in the absence of infection. Conversely it is now recognised that urinary infection may occur in the absence of pyuria, and even when pyuria is present the number of pus cells seen on microscopy depends on so many variables that it is of doubtful value even when the procedure is carefully standardised, and valueless if it is not. Among the factors to take into account are: the magnification used and the number of fields counted; whether or not the urine is centrifuged, the time since the urine was passed, the rate of urine flow, and the rate of production of pus cells. Loudon and Greenhalgh (1962) noted "again and again" that the degree of pyuria varied greatly in successive samples collected from the same patient over a short period of time. Nevertheless in their survey 78 per cent. of patients had 'obvious pyuria' and 22 per cent. had 'slight pyuria'.

Ambrose and Hill (1965) in a careful statistical study of 146 specimens from patients with pyelonephritis, but not necessarily containing a significant bacteriuria found that only half the specimens had more than three pus cells per high power field, and there did not appear to be any positive correlation between the presence of pus cells and presence of a significant bacteriuria.

In the survey carried out by Mond and his colleagues (1965) all the symptomatic bacteriuric group had over 10 pus cells per cubic mm (and 96 per cent. had over 50 per cu. mm) whereas 47 per cent. of the symptomatic non-bacteriuria group had over 10 pus cells per cu. mm, and only 6 per cent.

of the control non-symptomatic non-bacteriuric patients had that level of pus cells in their urine. Among the children of Mond's practice, however, there was no such correlation between pyuria and bacteriuria. Mond, Gruneberg and Smellie (1970), screened all but 10 of the 436 children under 13 in the practice. Of the 41 specimens of urine showing more than 10 pus cells per cu. mm. only one showed bacteriuria, and of the 5 children with bacteriuria only one also had pyuria.

In an examination of 205 consecutive urine specimens submitted from the Royal Aberdeen Hospital for Sick Children to the bacteriology laboratory, pyuria, defined as over 3 pus cells per high power field, was associated with significant bacteriuria in 8 cases but with a sterile urine in 4 cases. Conversely there were 18 cases of bacteriuria without pyuria, and 175 in which both examinations were negative (Hulbert, unpublished observations). Finally Brumfitt (1964) concludes in a paper entitled "Urinary cell counts and their value" that "Pyuria is not of significant value in the diagnosis of urinary tract infection".

Treatment of urinary infection in general practice

It is accepted as normal practice that most urinary tract infection in general practice should be treated without bacteriological control because most of the organisms are sensitive to the commonly used antibiotics, and also because of the difficulty in getting bacteriological results (Eykn and Phillips, 1969). Even those practitioners who do make use of the bacteriological facilities in their area generally treat the patient empirically, modifying or abandoning treatment in due course in the light of the report from the laboratory. Clearly when a large number of patients are being treated for a condition which nearly half of them may not have, a cheap,

non-toxic effective (and preferably bactericidal) antibiotic is required. For many years the sulphonamide group of drugs has been used in this situation, and they still enjoy considerable and justifiable popularity which has recently been enhanced by their combination with trimethoprim.

Mond et al. (1965) reports on the treatment of 43 episodes of urinary tract infection due to E. coli. Only one of the strains was resistant to 50 µg of sulphonamide and this patient was treated with tetracycline, as was one other patient with a history of sulphonamide sensitivity. The remaining 41 patients were given either sulphadimidine or a mixture of sulphonamides in a course lasting 8 days. Thirty six were clear at the end of treatment and two relapsed by the sixth week giving a cure rate of 83 per cent. at 6 weeks.

Other drugs, notably ampicillin, tetracycline, nalidixic acid and nitrofurantoin are suitable for use in general practice, but without exception they are much, or very much more expensive than the sulphonamide drugs (see Table 59), and some have other disadvantages.

Treatment of asymptomatic bacteriuria

Asscher et al. (1969) carried out a carefully controlled trial of the treatment of asymptomatic bacteriuria. A group of 49 asymptomatic bacteriuric non-pregnant women were given a course of nitrofurantoin. Those whose bacteriuria was not eradicated were given a course of ampicillin, the remainder being given a placebo, and none knowing the results of treatment. A control group of 45 women were given two courses of the placebo. After 6 months 59 per cent. of the treated group and 31 per cent. of the placebo group had sterile urine, and at one year the figures were 55 per cent. and 36 per cent. Statistically there was no significant difference between the groups.

When bacteriuria complicates pregnancy, however, treatment is more effective. Approximately 40 per cent. of patients with bacteriuria of pregnancy develope acute pyelonephritis of pregnancy and this can be prevented with adequate treatment, (Kass, 1960; Kincaid-Smith and Bullen, 1965; Little, 1965). Moreover the prematurity associated with bacteriuria can be almost eliminated with successful treatment (Kass, Savage and Santamarina, 1965).

Asymptomatic bacteriuria among children is probably quite rare. Savage et al. (1969) found an incidence of 2.1 per cent. of undiagnosed infection among 5-year-old school girls, but of the 20 positive children 18 either had a history of past infection, or complained of one or more of the following: enuresis, urgency, frequency, or unexplained fevers, and both children with no symptoms were at or below the tenth percentile for height on the Tanner and Whitehouse growth charts.

Value of screening tests for asymptomatic bacteriuria

If a screening procedure is to be successful it must firstly identify the disease before serious damage has occurred, and treatment must be available to cure or arrest the disease. On both accounts Asscher and his colleagues argue that screening for non-pregnant women fails. In adult women the disease is probably of long standing, and Asscher's own trial did not show that treatment produced significantly better results than spontaneous remissions (Asscher et al., 1969).

For pregnant women, however, Little (1965) calculated that screening might save about 21,000 cases of acute pyelonephritis of pregnancy. The cost of screening is not negligible at about £0.25 per examination (Asscher, 1970) but in this group of patients a saving of nearly £500,000

in England and Wales could result from the reduction in admission to acute hospital beds for pyelonephritis of pregnancy, as well as the reduction in expensive treatment for premature infants, in addition to an uncalculated (but small) saving in maternal and neonatal lives.

In children screening for bacteriuria at school entry (or earlier) may serve a different purpose in that it will identify those children with underlying renal abnormalities and those with symptomatic urinary infections. So far it is not certain whether screening will confer any benefit on the children but Hodson (1965) has made the encouraging claim that the growth of a kidney which is arrested by pyelonephritis is restored with treatment rendering the urine sterile. Also Normand and Smellie (1965) treated 66 children with urinary infection, showing radiological abnormalities, with long term antibiotic and found that the attack rate of clinical symptomatic urinary tract infection was reduced from 2.5 to 0.3 attacks per patient per year.

ABSORPTION AND EXCRETION OF PENICILLIN

Much work has been done on the absorption of oral penicillin G, on the blood levels achieved, and on its excretion. McDermott et al. (1946) conducted a particularly careful investigation. They found that between 10 and 32 per cent. of an oral dose of 315,000 units (190 mg) was excreted in the urine, compared with 60 - 100 per cent. of the same dose given intramuscularly. These figures were confirmed by Wright with a dose of 200,000 units (120 mg). Bunn (1950) and Linden, Finegold and Hewitt (1955) noted that penicillin levels did not increase in proportion to the size of the dose. Most penicillin is absorbed from the duodenum, the amount coming from the stomach, ileum and, probably the jejunum being small.

Effect of gastric acid on absorption

McDermott found that inactivation by gastric acid was variable but seldom great. If the pH was 2, or less, inactivation was rapid, but several hours were required for complete inactivation at pH values between 2 and 4, and with one or two exceptions he found that blood levels and urinary excretion of penicillin were similar in normal and achlorhydric patients. The work of Finland, Meads and Ory (1945) and Rammelkamp and Helm (1943a), however, contradicts these findings. They observed that achlorhydric subjects absorbed penicillin better than normal subjects. In the same study Rammelkamp and Helm carried out in-vitro studies on the effect of saliva, gastric and duodenal juices on penicillin. They found that penicillin was not inactivated by saliva, bile, duodenal or jejunal secretion but that it was inactivated by gastric juice at body temperature, and that the moiety of gastric juice responsible was the hydrochloric acid and not pepsin.

Acid protective agents

If gastric acid is not responsible for the inactivation of a large proportion of the dose of penicillin, acid protective or neutralising agents are not likely to improve penicillin absorption. Published reports confirm this. Free et al. (1944) tried giving penicillin with and without bicarbonate of soda, and McDermott et al. (1946) also tried an unspecified antacid, to reduce the pH of the gastric fluid. Both groups found that absorption of penicillin was best when taken alone, and indeed Free found that bicarbonate reduced absorption by more than 50 per cent. Free also noted that the time of maximum recovery was delayed from the first hour to the second. The reason for this delay may have been that the bicarbonate delayed the emptying of the stomach so giving the acid time to act.

In 1941 Abraham and his colleagues, who were concerned not to waste any penicillin, tried different methods of gastrointestinal administration. The use of magnesium trisilicate and enteric-coated capsules were tried unsuccess-:fully. The instillation of the penicillin through a naso-gastric tube passed into the duodenum was successful but too troublesome. Abraham noted that any enteric-coating must be timed to release the penicillin very soon after it passes through the pylorus or else the drug may pass by the duodenum where maximum absorption takes place. Finland and his colleagues (1945) found that commercial penicillin powder dissolved in saline was absorbed at least as well as any of the buffered, enteric-coated or otherwise-treated preparations.

Variability in penicillin absorption

Variability of absorption of the drug is a problem which has not been overcome or satisfactorily explained. The serum levels of penicillin achieved after oral therapy vary considerably; they are unpredictable, and

impracticable to assess (Lancet, 1954). However, the variability may have been overstressed. Twenty-nine subjects investigated by Wright (1955) all had levels of penicillin in the blood in excess of 0.05 units (0.03 µg) per ml 2 hours after an oral dose of 125 mg, and only one person out of 35 who were given an oral dose of 375 mg had an undetectable level of penicillin in the blood after 6 hours in Fairbrother and Daber's (1954) series. Furthermore, although there were wide differences in the serum levels, Foltz and Schimmel (1953) found that the difference in the actual amount of penicillin excreted in urine did not vary so widely.

Finland and his associates (1945) found that if the drug were given half an hour before the meal, absorption was good and regularly gave predictable serum levels. Peck and Griffith (1955) in a cross-over trial compared the levels of penicillin achieved when the drug was taken after a 550-calorie meal with the levels achieved with fasting subjects. Serum levels after food were only half as great, and were more variable, than those achieved with a fasting subject.

Henderson and McAdam (1946) noting that destruction of penicillin by penicillinase producing organisms in the jejunum might be another important factor limiting penicillin absorption found that, in neonates, penicillin blood levels were not only regular and predictable, but were maintained at or above a therapeutic level for twice as long as the same dose given intra-muscularly. This they attributed to relatively little inactivation by gastric juice, which is not highly acid in neonates, and to the absence of penicillinase producing organisms in the jejunum, permitting absorption to take place over a longer time, and more completely.

Several workers have calculated oral-parental ratios. McDermott et al. (1946) considered that 5 times the parenteral dose administered orally would give similar serum levels in practice, whereas Finland and his colleagues (1945) considered that not less than 2½ times the parenteral dose would be adequate.

Absorption of other penicillin compounds

The makers of some other penicillin compounds, as opposed to acid protected potassium penicillin G, have claimed that their products are not susceptible to the inconsistencies of absorption which affect penicillin G. In particular this has been claimed for benzathine penicillin (N : N'-dibenzylethylenediamine penicillin) but the variable reports from several trials of the drug (Bayne et al., 1953; Cathie and MacFarlane, 1953; Welch, Randall and Hendricks, 1953; Wright et al., 1953) indicate that these claims may be lacking in support. In fact in Fairbrother and Daber's (1954) series, potassium penicillin G was more reliably absorbed than benzathine penicillin. Foltz and Schimmel (1953) note in their comparison of orally administered procaine penicillin G, potassium penicillin G, and dibenzyl dipenicillin G that the absorption by a single patient of each of the penicillins was in the same category, i.e. if one was well absorbed, all were.

Blood levels of oral penicillin G

Absorption of penicillin from the gastro-intestinal tract is rapid, and maximum blood levels are achieved in 30 - 60 minutes, and thereafter the disappearance is rapid and is a reflection of the maximum height achieved and is not significantly affected by continued absorption, which is slight, McDermott et al. (1946). Putnam et al. (1955) found that the mean blood levels in 116 subjects after an oral dose of 200,000 units (125 mg) fell

from 0.578 units (0.36 µg) after one hour to 0.003 units (0.002 µg) after 8 hours, and in another group of 48 subjects given the same dose the amount of penicillin per ml of blood fell from 0.699 units (0.435 µg) to 0.003 units (0.002 µg) over the same time scale. Symon (1955) found that blood levels following 400,000 units of oral penicillin varied from 0.5 to 0.3 units (0.3 to 0.18 µg) per ml over 6 hours, with a mean of about 0.2 units (0.12 µg).

Linden, Finegold and Hewitt (1955) compared the absorption of oral penicillins G and V and found that with a dose of 400,000 units (250 mg) penicillin G was better absorbed giving earlier and more prolonged blood levels, but with an increase of the dose to 1,000,000 units (600 mg) little increase in the penicillin blood levels was apparent so that penicillin V became the better absorbed of the two drugs.

Distribution of penicillin in the body

Once in the body penicillin is widely distributed. Of the penicillin in the blood about 60 per cent. is bound to plasma proteins. Levels in the eye fluids, C.S.F. and joint fluids are lower than those in the blood, and also in the precordial and pleural fluids, but levels in the peritoneal fluid are higher, (Rolinson and Sutherland, 1965; Friend, 1966). Penicillin is actively removed from the C.S.F. by a mechanism analagous to that which exists in the proximal tubule in the medulla of the kidney. This transport mechanism is impaired by probenecid (Fishman, 1966).

Excretion of penicillin

Some of the absorbed penicillin is excreted in the bile (Rammelkamp and Helm, 1943b), but over 80 per cent. is excreted by the kidney, the rate of disappearance from the blood being similar to that following parenteral

administration of the drug (McDermott et al., 1946). Eagle and Newman (1947) found that after an intramuscular injection 60 per cent. of the dose appeared in the urine in the first hour, and that the level of penicillin in the blood fell off thereafter at an average rate of 70 - 80 per cent. per hour, or 2 - 3 per cent. per minute. Of the penicillin excreted by the kidneys 90 per cent. is excreted by the tubules and 10 per cent. by the glomeruli, and a healthy kidney is capable of excreting 3,000,000 units (1.8 g) per hour (Friend, 1966). Eagle and Newman found that renal clearance of penicillin approximates to the total renal plasma flow, and is independent of the blood level (from 0.05 to 10 µg per ml) and of the urine flow. The only way to limit excretion is to limit renal blood flow.

Peeney (1947) carried out an investigation in which he correlated the penicillin sensitivity of 138 Gram-negative bacilli isolated from urines with the levels achieved in the urine during parenteral or oral therapy. Of 81 strains of Escherichia coli 49 were sensitive; 22 of 42 strains of Proteus, each of two paracolon strains, and none of 13 strains of Ps. aeruginosa were sensitive to 200 Oxford units (125 µg) of penicillin per ml. The mean penicillin level in the urine for 3 hours after an intramuscular injection of penicillin increased from 12 units (8 µg) per ml to 85 units (50 µg) per ml as the dose was increased from 5000 to 50,000 units (3-30 mg). When a continuous drip of penicillin was given at a rate of 400,000 units (250 mg) per 24 hours the urine level was 256 units (150 µg) per ml. When 100,000 units (60 mg) of penicillin was given intramuscularly a level of 680 units (410 µg) per ml was recorded in the urine one hour later. It is worth noting that all these levels were measured in cases suffering from

urinary tract infection, and that the effect of any penicillinase produced by the infecting organism is not mentioned, and presumably not accounted for.

Urine levels of penicillin G after oral therapy

Many of the investigations cited above required the measurement of the concentration of penicillin in the urine, but in few was it actually quoted. In fact only one original reference to the level of penicillin G obtained in the urine at intervals after an oral dose could be found even after a careful search of the literature assisted by the librarians of several of the manufacturers of penicillin. Peeney in the course of investigations mentioned earlier carried out a single experiment on one subject who was given 90,000 units (55 mg) orally. The levels in the urine (read off a graph) were: 43 units (25 µg) per ml after 1 hour, 22 units (12 µg) per ml after 3 hours, and 10 units (6 µg) per ml after 12 hours. No information about bladder emptying or urine volume was given, but fluid intake was restricted to 3 pints per 24 hours.

Garrod and O'Grady (1968 page361) mention a level of 250 µg per ml as attainable in the urine presumably after systemic therapy, and Barber and Waterworth (1964) consider that 60 µg per ml was likely to be achieved after systemic therapy. Thomas and Lavine (1945) working 20 years earlier and using very much smaller doses of penicillin reported that 50 - 300 Oxford units per ml (30 - 180 µg) were easily obtainable in the urine after systemic therapy.

However, if one assumes that 10 per cent. of an oral dose is excreted in the first six hours (Wright et al., 1955), and that urinary excretion is 1200 ml per day, then the average concentration in the urine in the first 6 hours following an oral dose of 500 mg would be over 160 µg per ml, with

peak levels much in excess of this figure. Vinnicombe (1966) reporting on some of the work carried out by Stamey in California notes that urine levels of 150 µg per ml were achieved after an oral dose of 500 mg, and he mentions that 80 per cent. of strains of E. coli, and 91 per cent. of strains of proteus isolated from 320 cases of urinary infection were sensitive to this level.

Preparations of penicillin G marketed at present

A number of different preparations of penicillin G are currently available for oral administration. These include potassium penicillin G, procaine penicillin G and benzathine penicillin G. Also available are some enteric-coated preparations of potassium penicillin G of which Fallapen (BDH Pharmaceuticals Ltd.) and Hyasorb (Berk Pharmaceuticals Ltd.) are representative. Fallapen consists of an inner core of potassium penicillin G protected by an enteric capsule and an outer layer for immediate absorption. Hyasorb consists of granules of penicillin, some uncoated, others with different thicknesses of enteric coat, so that the penicillin will be absorbed over a period of time and, the makers claim, maintain therapeutic serum levels (for sensitive Gram-positive bacteria) for up to 8 hours after one dose.

A third type of penicillin G preparation is penamicillin (Havapen, by John Wyeth and Brother Ltd.). This is an acetoxymethyl ester of penicillin G which is hydrolysed to penicillin G in the body. (Probably this occurs as it is absorbed through the mucosa of the gastro-intestinal tract). It is claimed by the manufacturers that it is absorbed better than penicillin itself. In Table 1 the results of 8 investigations into the absorption and blood levels of these preparations are abstracted and tabulated.

TABLE 1

Concentration of penicillin in blood serum after oral administration of various types of penicillin: results abstracted from eight published sources

Source. For key see over	Type of penicillin	Dose (units)	Number of patients	Mean blood levels (units per ml) at different times after oral dose					
				1	2	4	6	8	12
1	Fallapen	500,000	10	0.458	0.384	0.324	0.270	0.197	0.112
2	Fallapen	500,000	24	0.420	0.377	0.286 after 5 hours		0.155	0.054
3	Benzathine	300,000	12	0.192	0.135	0.075	...	...	...
3	Procaine	300,000	12	1.04	0.464	0.134	...	...	...
3	Potassium	300,000	12	1.19	0.550	0.177	...	...	...
4-1	Benzathine	300,000	6	...	0.3	0.09	0.03	...	...
4-2	Benzathine	600,000	6	...	0.25	0.09	0.05	...	...
4-3	Benzathine	300,000	6	0.25	0.25	0.07	0.03	...	...
4-4	Potassium	300,000	6	0.5	0.35	0.15	0.03	...	...
5-1	Hyasorb	500,000	21	1.0	0.7	0.26	0.097	0.047	0.01
5-2	Hyasorb	500,000	10	0.48	0.44	0.26	0.10	0.07	0.01
5-3	Potassium	500,000	19	1.40	0.42	0.09	0.019	0.007	...
6	Hyasorb	500,000	45	2.10	1.70	0.24 after 3 hours	0.38	0.10	0.15
7	Benzathine	300,000	10 - 20	...	0.076	...	0.05	...	...
7	Potassium	300,000	28 - 21	...	0.32	...	0.08	...	...
7	Benzathine	600,000	68 - 59	...	0.17	...	0.06	...	...
7	Potassium	600,000	58 - 35	...	0.46	...	0.16	...	...
8	Pen-amicillin	400,000	20	0.325	0.298	0.231	0.111	0.089	0.021

Source references and notes pertaining to Table 1

(1) Crignon and Leboef (1958). In the first eight hours all 10 patients had measureable concentrations of penicillin in the blood, none being less than 0.03 units (0.02 µg) per ml.

(2) Ballon et al. (1958). In the first eight hours the lowest recorded concentration of penicillin in the serum was 0.025 units (0.015 µg) per ml, but there were wide variations in the results.

(3) Foltz and Schimmel (1953). This was a cross-over study with the same 12 subjects. Urine recovery of penicillin was measured and was: benzathine penicillin - 6.7 per cent. of the dose; procaine penicillin - 24.8 per cent. of the dose; potassium penicillin - 33.3 per cent. of the dose.

(4) Bayne et al. (1953). Different patients took part in experiments 4-1 and 4-2. Experiments 4-3 and 4-4 involved the same 6 subjects in a cross-over study.

(5) Brown and Brown (1964). Experiments 5-1 and 5-2 were similar but separate trials. Up to six hours after administration of the drug all but one patient in both groups had a measureable concentration of penicillin in the blood serum. With potassium penicillin G (experiment 5-3), although the concentration of penicillin in the blood was high at first, it declined rapidly so that even two hours after administration of the drug one patient had an unrecordable concentration of penicillin in the serum.

(6) Beck, Barach and Rose (1957). A complicated trial. Some of the patients were fasting and some were not at the time of administration of the drug. It seemed that absorption was better among fasting patients, but there were wide differences between individual results.

(7) Fairbrother and Daber (1954). This was not a cross-over trial. The first figure in the 'number of patients' column represents the number of subjects who had blood penicillin levels taken at "1 - 3" hours after administration, and the second is the number who had penicillin levels measured at"5 - 6"hours after the drug was given.

(8) Stewart (1967). The urine recovery of penicillin following penamicillin administration (to different patients from those whose blood penicillin concentrations were measured) was 20 per cent. of the dose.

IMPORTANCE OF URINE LEVELS OF ANTIBIOTIC

Stamey and Peau (1960) in a very careful study of a single normotensive patient with a unilateral pyelonephritis demonstrated that the pyelonephritic lesion is confined to the medulla of the kidney. This patient had had recurrent urinary infections, the latest episodes being caused by a strain of Ps. aeruginosa. These had been successfully treated by colistin methane sulphonate but recurred within a few days of cessation of treatment. In his studies Stamey catheterised both ureters and compared the function of the diseased kidney with that of the normal contralateral kidney, and found that the defect was manifest by a marked loss of water and sodium, indicating that the lesion was in the medulla where water and sodium are normally reabsorbed.

An operation to remove the diseased kidney was planned, and for 48 hours before it the patient received 75 mg of colistin methane sulphate 12 hourly. At operation the kidney was removed carefully, and several grams of the cortex were removed for bacteriological analysis, and also several grams of medulla. The cortex was found to be sterile, but the medulla contained over 1200 bacilli (Ps. aeruginosa) per gram, in spite of the treatment (to which the organisms were sensitive) and in spite of there being a sterile urine.

Reabsorption of weak acids and bases

Milne, Scribner and Crawford (1958) have shown that weak acids and bases are reabsorbed from the glomerular filtrate in the distal tubule. They also showed that this was a physico-chemical diffusion process which was affected by the state of the acid-base balance of the body and the pH of the urine. Woodruff, Malvin and Thompson (1961) confirmed this work using stop-flow studies on the nitrofurantoin in anaesthetised dogs. They demonstrated a re-absorption gradient similar to and overlapping that for sodium.

Nitrofurantoin is a weak acid and so in alkaline urine there is a greater degree of ionisation, less re-absorption and a higher concentration than in acid urine. This fact may explain some of the variability in the results noted in the treatment of pyelonephritis with nitrofurantoin (Richards, 1955).

Stamey and his associates (1965) consider that it is likely that the concentration of antibiotics (which are weak acids or bases) in the collecting ducts of the tubules are, by virtue of the fact that they are being re-absorbed from the tubule, transferred to the interstitial water of the medulla. Moreover, since only a small fraction of the total renal blood flow perfuses the medulla (Thornburn et al., 1963) the contribution to the interstitial water by the blood is likely to be small.

Renal tissue levels of antibiotics

Attempts to assay the level of antibiotics in the renal tissue or in the interstitial water of the kidney do not confirm Stamey's hypothesis. Direct techniques whereby a portion of the kidney tissue is ground up and assayed for its antibiotic level are subject to considerable errors if even a small amount or urine is included in the homogenate. Jameson (1965) analysed the renal cortical and renal medullary levels of nalidixic acid in patients undergoing nephrectomy. However, in the presence of high urinary levels of the drug it is impossible to accept without reservation his conclusion that the majority of patients had renal tissue levels significantly greater than the plasma level.

A different type of investigation has been the use of antibiotics tagged with radioactive isotopes or fluorescent dyes. Such techniques have been used to study the distribution in the kidney of tetracycline (Malek et al., 1963; Helander and Bottiger, 1953), cephaloridine and nitrofurantoin (Currie, Little

and MacDonald, 1966) and streptomycin and dihydrostreptomycin (André, 1956). Considerable differences between antibiotics have been noted. The tetracyc-:lines have been shown to concentrate in infected tissue, although hydronephrosis greatly reduced the extent of this concentration. Nitrofurantoin was shown to localise in the lumen of the tubules and in the interstitial spaces of the medulla, but not within the cells. Cephaloridine was concentrated in the cells of the proximal tubule, whilst streptomycin and dihydrostreptomycin were present only in low concentration in these cells. Although these studies are useful and indicate different metabolic pathways for the different drugs, they do not permit a comparison between the level of an antibiotic in the kidney with the mean inhibitory concentration of an organism.

Antibiotic in renal lymph

In recent years two independent groups of workers have been measuring the concentration of antibiotics in the lymph draining from the kidney, and comparing it with blood and urine levels of the same antibiotics. There are two routes by which lymphatics drain from the kidney. The cortical lymphatics drain into the capsular lymphatics, whilst the medulla is drained into the hilar lymph vessels, one of which usually crosses the renal artery and is accessable forcannulation with a fine nylon catheter of 0.5 mm internal diameter, (Rawson, 1949; Narath, 1951; Katz, Cockett and Moore, 1964; Rhodin, 1964; Cockett et al., 1968). Renal lymph is said to arise both from renal tubular fluid and from renal blood plasma (Kapalan, Friedman and Kruger, 1942), but in constitution it closely resembles arterial plasma in its albumin globulin ratio (total protein is 2.76 g per cent., Mayerson, 1963), and in its concentrations of urea and electrolytes, its osmolality and total CO_2, (Keyl et al., 1965).

The function of the renal lymph is to return to the circulation the proteins and fluid that leak from the capillary blood vessels into the interstitial spaces of the kidney (Kapalan et al., 1942), and to balance intrarenal fluid volume and regulate its composition. In view of the but slight differences between capsular and hilar lymph in urea and electrolyte concentrations referred to above, and borne out by the experiments with antibiotics to be considered later, the contribution to the hilar lymph by fluid from the renal tubules must be small. Dock (1953) compared the renal tubules with a leaky hose allowing extravasation of glomerular filtrate into the interstitial spaces. If this is so then most of the fluid must be returned to the tubule because it does not appear to be drained by the hilar lymphatics.

Cockett and his colleagues in Los Angeles, and Chisholm from the Post-graduate Medical School, London, have in recent years published several reports on the concentrations of a variety of antibiotics in renal lymph. The technique used by both groups was basically the same (Chisholm et al., 1968; Cockett et al., 1965). Dogs were anaesthetised and the kidney exposed. The capsular lymphatics were easily identified, but Cockett found that the hilar lymphatics required some dissection and an incision of the peritoneum, and he could cannulate successfully the hilar lymphatics in only one dog in three. In some animals the thoracic duct and, or the cysterna chyli were also cannulated.

The rate of flow from a renal lymphatic rarely exceeded 0.1 ml per minute so only a few estimations of antibiotic in renal lymph could be made. Blood and urine collections were made at the mid point in time of the lymph collection.

The results of some representative experiments are summarised in Table 2. Where the results have been given as histograms or graphs the figures have been

TABLE 2

Levels of antibiotic in lymph, plasma and urine of experimental dogs

Drug and dose	Route	Time (hr) after drug given	Level (µg per ml) of drug in					Reference*
			Urine	Plasma	Lymph			
					Hilar	Capsular	Other (Specify)	
Nitrofurantoin	Oral	1	-	7	10	8	-	1
5 - 7.5 mg/Kg	Oral	4	-	3	8	6	-	1
	Oral	3	-	0.5	3	-	1 (Cysterna Chyli)	3
	Oral	3	-	0.5	2	-	0.25 (ditto)	3
	I.V.	1	-	4	-	-	11 (renal)**	4
	I.V.	1	-	4.5	-	-	4 (renal)**	4
Chloramphenicol	Oral	1	-	19	-	-	13 (renal)**	1
50 mg/Kg	Oral	3	-	8	-	-	4 (renal)**	1
Penicillin G	I.V.	1	125	10	-	8	-	2
12 mg/Kg over 4 hours	I.V.	3	525	12	9	-	-	2
Cephalothin 46 mg/Kg	I.V.	3	500	60	50	-	-	2
Cephaloridine ? dose	I.V.	2	650	10	20	-	-	2
	I.V.	3	800	8	10	-	-	2
Naledixic acid	Oral	3	-	7	5	-	-	2
50 mg/Kg	Oral	3	150	12	-	-	5 (renal)**	2
Cycloserine	I.V.	3	300	60	-	-	60 (renal)**	3
50 mg/Kg	I.V.	3	750	25	-	-	30 (renal)**	3
Tetracycline	I.V.	2	130	11.6	10.6	-	-	3
48 mg/Kg	I.V.	3	105	15.4	8.1	-	-	3
Gentamicin	I.V.	-	-	6	-	-	4 (renal)** 4 (thoracic)	4
1.5 - 2 mg/Kg	I.V.	-	-	3.5	-	-	3 (renal)** 3.25 (thoracic duct)	4
Carbenicillin	I.M.	2	-	10	-	-	10 (thoracic)	4
20 mg/Kg	I.M.	2-4	-	9	-	-	6 (thoracic)	4

* See note 1, ** See note 2, on next page.

Table 2 (continued)

Note 1. * Reference: 1 Cockett *et al.*, 1965.

2 Cockett *et al.*, 1967.

3 Cockett *et al.*, 1968.

4. Chisholm *et al.*, 1968.

Note 2. ** renal: Unspecified renal lymph.

read off and tabulated. Nitrofurantoin was the first drug to be investigated in this way (Katz et al., 1964), and most, but not all of the dogs had higher concentrations of antibiotic in the lymph than in the plasma, and slightly higher concentrations in the hilar lymph than in the capsular lymph. In none of the dogs was the hilar lymph level more than 3 times the plasma level, and in a few animals it was less than the plasma level. Similar results have been obtained for cephaloridine, but not for tetracycline, cycloserine or chloramphenicol where there seems to be little difference between the plasma and hilar lymph levels, or for nalidixic acid, carbenicillin and gentamicin where the plasma levels were in general higher than the hilar lymph levels.

Cockett and his colleagues (1965) measured the plasma and hilar lymph concentrations of nitrofurantoin in a patient with hydronephrosis who was urdergoing pyeloplasty. One hundred and eighty mg of the drug was given intravenously over a 2 hour period and the levels measured in hilar lymph and plasma. After 1 hour the plasma level was 3 µg per ml and the hilar lymph level was 16 µg per ml, and after 2 hours the levels were 3.5 and 14 µg per ml respectively.

The conclusions that the two groups of investigators came to are complementary. Chisholm (1968) making the assumption that the renal lymph levels approximate to the levels in the renal parenchyma writes: "The fact that a drug is concentrated in the urine does not imply that the adjacent renal parenchyma has the same or even a similar concentration; the present studies show that the lymph levels in the thoracic duct and kidney were entirely unaffected by high urine levels". Cockett (1965) concludes, "All therapeutic agents alleged to be effective in treating pyelonephritis need to be re-evaluated by the renal lymph-plasma system".

However there must be caution before accepting these conclusions which are not confirmed by the undoubted clinical success of nalidixic acid and nitrofurantoin. In man the normal dose of nitrofurantoin is 100 mg three or four times a day. This about one fifth of the dose per kilogram given to the dogs. With this dose a serum level of 0.5 to 1 µg per ml is achieved, and even the most optimistic interpretation of the results summarised Table 2 would give a hilar lymph level of no more than 3 µg per ml. The urine level with this dose varies widely, but a mean level of 100 to 150 µg per ml could be expected. The mean inhibitory concentration of nitrofurantoin for 'sensitive' strains of E. coli is about 15-50 µg per ml. Quite clearly nitrofurantoin could not be successful in treating pyelonephritis unless the urine concentration were significant, or unless there were other factors involved.

It may be that antibiotics penetrate into the renal lymph more effective-:ly in the presence of infection and inflammation. Whilst this happening might preserve the hypothesis that renal lymph concentrations of antibiotic are significant in urinary infection and ought to exceed the minimum inhibitory concentration of the infecting organism, the extent of the alteration in the lymph concentrations of antibiotic would need to be so great that it would make the measurement of this level in healthy animals irrelevant.

PENICILLIN SENSITIVITY OF GRAM-NEGATIVE BACILLI

Penicillin compounds with different properties are now numerous, and it is commonplace to consider the therapeutic use of one or more of this group of drugs for most of the bacterial infections that afflict mankind. Abraham and his colleagues (1941) noted that 1000 times the concentration of penicillin required to inhibit the Gram-positive cocci was needed for the Gram-negative bacilli. Helmholtz and Sung (1944a) noted that urine from patients receiving 40,000 - 100,000 Oxford units (24 - 60 µg) of penicillin G per day by injection did contain sufficient penicillin to be bactericidal for many Gram-negative bacilli. They studied 73 strains of Gram-negative bacilli isolated from cases of urinary tract infection, and they found that 30 units (18 µg) of penicillin per ml of urine was bactericidal to all 13 Proteus strains investigated, and to 41 per cent. (16) of the 39 strains of E. coli, but that it was not effective against the three strains of Ps. aeruginosa or the 18 strains of Aerobacter aerogenes. They recognised the division of strains of E. coli into two groups, those sensitive to the concentrations of penicillin attainable in the bladder and those more highly resistant.

Thomas and Levine (1945) and Stewart (1945) measured the sensitivity of 34 strains of Gram-negative bacilli including strains of E. coli, Proteus, Salmonella, Shigella and Aerobacter, and they found that all but three of them were sensitive to 100 Oxford units (60 µg) of penicillin per ml, and they noted that these levels could be achieved in the urine and other body fluids. Coleman and Taylor (1949) measured the penicillin sensitivity of 125 urinary pathogens, and found that 55 of 61 strains of E. coli (90 per cent.) were sensitive to 100 Oxford units (60 µg) of penicillin G, all 20 Proteus vulgaris strains, 2 of the 6 paracolon strains and the single Alkaligenes faecalis strain were also

sensitive to this level of penicillin, whilst none of the 19 strains of Aerobacter aerogenes or 14 strains of Proteus morgani or three strains of Ps. æruginosa were penicillin-sensitive. Of the 46 strains resistant to 100 Oxford units (60 µg) of penicillin G, all but 8 were also resistant to 500 Oxford units (300 µg). It might be noted that in this and in other papers reporting penicillin sensitivity at this time the 'inoculum effect' was not known, and the standard inoculum for tube dilution sensitivities was one drop of an overnight broth culture of the organism. Although the 'inoculum effect' is less important with Gram-negative bacilli than with Gram-positive bacteria (Percival, Brumfitt and de Louvois, 1962), the penicillin sensitivity of the same strains carried out by modern methods (Cruickshank, 1969, page 901) might be considerably lower.

In 1946 Duguid demonstrated that all Gram-negative bacilli, even Ps. aeruginosa, were sensitive to penicillin provided the concentration of the drug was high enough (up to and exceeding 10,000 units (6 mg) of penicillin per ml in some cases) and that the mechanism of action of penicillin on the sensitive and resistant organisms was essentially the same.

Use of penicillin confined to Gram-positive bacteria

When penicillin was scarce it was natural to confine its use to those cases where it could be given economically, and these were predominantly coccal infections. Moreover most Gram-negative urinary infections were susceptible to treatment with mandelic acid or the sulphonamides, and only those caused by Streptococcus faecalis, which is resistant to both these drugs, were considered for treatment with penicillin (Helmholtz and Sung, 1944b). Then with the discovery of tetracycline and chloramphenicol, both of which were active against Gram-negative bacilli at levels that could readily be achieved

in the tissues the position of penicillin as a Gram-positive and coccal antibiotic was re-inforced, and throughout the 1950's interest was centered on prolonging the action of a single dose of penicillin and on improving its acid stability and absorption.

Production of ampicillin

With the isolation of the penicillin nucleus (Batchelor et al., 1959, 1961) and the production of ampicillin (Rolinson and Stevens, 1961), the emphasis changed and attention was focussed on the manufacture of the semi-synthetic penicillins, and on widening the spectrum of action of the penicillin groups of drugs. Studies comparing the action of different penicillins on a variety of organisms were carried out, and this work indicates that most strains of E. coli isolated from urinary tract infections are sensitive to 12.5 to 50 µg of penicillin G per ml (Rolinson and Stevens, 1961; Sutherland, 1964; Garrod and O'Grady, 1968), and that most strains of Proteus isolated from clinical material are sensitive to 8 µg per ml of penicillin (Barber and Waterworth, 1964). Ps. aeruginosa, however is usually insensitive to levels of 500 µg of penicillin or more (Sutherland, 1964).

Comparison of penicillin G with ampicillin

Sutherland (1964) compared the action of seven penicillins (ampicillin, penicillin G, penicillin V, phenethicillin, propicillin, methicillin and cloxacillin) against a variety of Gram-negative bacteria. In general the strains fell into two groups, those that were sensitive to 1.25 - 5 µg of ampicillin per ml, and these were also sensitive to 2.5 -25 µg of penicillin G per ml, and those strains which required 125 µg of ampicillin per ml or more for inhibition, and these strains were generally resistant to 500 µg of penicillin G. Ampicillin was 2 - 5 times more active (weight for weight)

than penicillin G except against Aerobacter aerogenes, which, although relatively resistant to both drugs, was more susceptible to penicillin than to ampicillin.

Taking account of penicillinase production Sutherland divided his strains into three groups.

(1) Those that produced little or no penicillinase and which were sensitive to ampicillin and penicillin G. These included some strains of Salmonellae, the strains of Proteus mirabilis, and Klebsiella.

(2) Those that caused little or no destruction of ampicillin, but a more marked destruction of penicillin G, and so were relatively sensitive to ampicillin, but resistant to penicillin G. Strains of E. coli and Shigella were in this group.

(3) Those strains that were capable of destroying both drugs. This group was further divided into -

(3a) those that were intrinsically sensitive, including some strains of Aerobacter aerogenes, and Proteus vulgaris and morgani and

(3b) strains that were intrinsically insensitive, e.g. Ps. aeruginosa and some strains of E. coli and Proteus mirabilis.

Ampicillin was found (by Sutherland) to be relatively stable to the penicillinase enzymes produced by certain of the Gram-negative bacilli, whereas penicillin G appeared to be ineffective against all penicillinase producing organisms.

Comparison of penicillin G with other penicillins

All the strains of bacilli in Sutherland's investigation (1964) were more resistant, and some much more resistant to the other penicillins tested (penicillin V, phenethicillin, propicillin, methicillin and cloxacillin) than

to penicillin or ampicillin. Barber and Waterworth (1962) carried out a similar investigation with eight different penicillins (ampicillin, penicillins G and V, phenbencillin, methicillin, and three isoxazolyl penicillins), and with phenyl-acetyl-amino-cephalosporanic acid thiouronium. They found that for Gram-negative bacilli, ampicillin was 4- 8 times as active as penicillin G, and that all the other penicillins were much more resistant. Garrod (1960) found that penicillin G was 8 - 16 times as effective against Gram-negative bacilli as was penicillin V. Stamey et al. (1965) found that of 94 strains of Gram-negative bacilli (40 of them E. coli) only three were lysed by 250 µg per ml of oxacillin, a further 9 by 500 µg, and for the remaining 82 strains even 500 µg per ml of oxacillin was ineffective.

The mean urinary excretion of a single dose of 500 mg of cloxacillin in 38 subjects was 48 per cent. in an investigation carried out by Knudsen (1962), and the proportion of a dose of penicillin V recoverable in the urine is only 25 per cent. (Heatley, 1956). Clearly the slightly higher urinary antibiotic levels that might be achieved with these drugs, compared with penicillin levels, would not compensate for the much greater resistance to the drugs of most Gram-negative bacilli.

Ampicillin, on the other hand, is not only more active than penicillin G against Gram-negative bacilli, but it is better absorbed, 30 per cent. of a single 500 mg dose being excreted in the urine in the first six hours, and titres of ampicillin in the urine rose to 1000 µg per ml with such a dose Rolinson and Stevens, 1961). However the effect of penicillin G is best exerted by a concentration of 5 - 10 times the mean inhibitory concentration of the infecting organisms, and no increase above this level will accelerate the effect, and indeed with strains of Staph. aureus and Str. faecalis such an increase

actually reduces the effect (Eagle, 1951). It might be therefore that the only advantage to accrue from the use of ampicillin against penicillin would be a reduction in the dose, or less frequent administration.

ABTIBIOTIC SENSITIVITY DISKS

The World Health Organization Report (No. 210) on the methods used to carry out sensitivity tests notes that those methods suitable for carrying out surveys or other research purposes are usually too laborious to use in a busy laboratory doing clinical bacteriology, especially where each organism may have to be tested simultaneously against several antibiotics. For this situation the report prefers commercially prepared filter-paper disks impregnated with the antibiotic.

The report notes that the amount of antibiotic in each disk depends on three factors: (1) the activity of the antibiotic; (2) the rate at which it diffuses into the medium; (3) the concentrations attainable in vivo and the therapeutic activity of the antibiotic in different situations. Furthermore it recommends special high concentration disks for urinary tract infections, noting the higher concentrations obtainable in the urine.

COMPUTER

The results of some of the experiments and surveys in this thesis have been analysed by a computer.

Computers are being used increasingly in many branches of pathology and clinical medicine. In most they merely process the data, but in clinical chemistry and haematology they may be linked to automated machines which carry out the full examination of the specimen and issue the report (Bernard et al., 1969; Neil and Doggart, 1969; Nelson, 1969). Semi-automated machines for scanning cervical smears will be possible in the near future (Dawson et al., 1969). In bacteriology, although some processes may be automated, the interpretation of culture plates and slides is likely to remain in the province of the bacteriologist for many years to come. The computer may be used, however, to process the results of examinations more quickly and to present them to the clinician more acceptably, communicating more information than was previously possible, and moreover the computer may introduce a useful aspect of quality control, and it will have research functions as well.

O'Brien, Kent and Medeiros (1969) in a useful paper on the analysis of sensitivity results, and their presentation to the clinician note , "Antimicrobial sensitivity tests of bacterial isolates from an infected patient often guide the selection of antibiotics for that patient. The accumulated results of all such testing in a hospital can also be expected to contain more general information useful to the physicians who practice there". Stirland, Hillier and Steyger (1969) gave other examples of the type of analysis of bacteriological information which might usefully be issued to clinicians and bacteriologists.

KINETICS OF URINARY TRACT INFECTION

In recent years considerable attention has been devoted to a study of the kinetics of the urinary tract. O'Grady and Cattell have discussed the general theory of the subject in considerable detail in two papers dealing with the upper urinary tract (1966a) and the bladder (1966b). It is necessary to distinguish between the upper urinary tract, comprising the kidney and ureters, and the bladder because conditions of bacterial growth differ considerably between them.

Kinetics of upper urinary tract infection

Bacteria multiplying in a normal upper urinary tract are existing in a continuous cultivation system with new medium being added, and the culture being drained off at the same rate. In this system the relationship between the 'perfusion volume ratio' (which can be expressed:

$$\frac{\text{urine flow rate}}{\text{volume of the system}}$$

and which is a measure of the rate of change of urine) and the rate of bacterial multiplication is critical. Since the maximum rate of multiplication of bacteria in urine is known and constant for a given set of conditions, the perfusion volume ratio that can just balance it can be calculated. A greater perfusion volume ratio (i.e. more urine secreted or a reduction in the volume of the system) will result in the number of organisms per ml in the urine gradually falling away to zero, whereas a decrease in perfusion volume ratio will result in an increase in the number of organisms per ml to the 'climax number' at which the multiplication rate will fall off to that just sufficient to use up the incoming medium.

The effect on this theoretical situation of unequal perfusion of different parts/

parts of the kidney, of cul-de-sacs producing areas of under-perfusion, and of sedimentation of debris into dependent parts of the system are discussed. Clinical examples are cited, and the importance of these complex considerations in urosurgical procedures are emphasized.

Kinetics of bladder infection

In the bladder the system is complicated because the volume of urine is continually changing. The rate of increase of the volume of the bladder and the frequency of micturition are to some extent under the control of the patient. Voiding from an infected bladder, moreover, cannot completely clear the bladder of organisms because of the residual urine, which may be as little as 0.5 ml in the male (McGregor and Wynne Williams, 1966), and because a film of infected urine is left on the walls of the bladder. (The bladder wall however, can probably destroy these organisms; Cox and Hinman, 1961; Vivaldi et al., 1965).

Two factors influence the total number of organisms left in the bladder: the frequency of bladder emptying and the residual volume. Clearly there is a critical relationship between these two factors. Assuming that only one organism has been introduced into the bladder and has started to multiply, if voiding is sufficiently infrequent and the residual volume large enough, then theoretically the number of organisms will increase to a level (10^5 per ml) at which infection is said to be present. If on the other hand voiding is more frequent, and or the residual volume is sufficiently small, the organisms will be washed out of the bladder and will not achieve significant numbers. The period of sleep is particularly important because it is a time when urine secretion is usually depressed, the urine concentrated (and so providing ample nutrient material) and micturition infrequent so that such organisms that remain in the bladder have a long period of time under optimal conditions in which to grow.

Other factors are also important. Although the number of organisms in the bladder rises steadily after micturition the concentration per ml of urine falls initially because the organisms are diluted by the fresh urine (assuming that the ureteric urine is bacteria free). The extent and duration of this fall depends on the rate of urine secretion and on the residual volume, and the authors show that doubling the rate of secretion has the same effect as halving the residual volume. Therefore the volume of residual urine must always be considered in conjunction with the urine flow rates. Less important factors also influence these theoretical considerations. Chief among these are urine composition, incomplete mixing, alterations to the rate of bacterial multiplicat-:ion as climax populations are approached, the absence of good aeration, and the antibacterial effect of the bladder if this property exists.

By giving a patient large quantities of water to drink (e.g. 300 ml per hour) the rate of urine flow is increased, frequent micturition is stimulated, and the urine is diluted. These represent the optimum conditions to achieve a washout.

Finally the authors recall the 'inoculum' effect, which is the decreasing effect of an antibacterial drug with increasing initial concentration of bacteria. Therapy should therefore be arranged so that maximum concentrations of the antibacterial agent are entering the bladder when the concentration of the organisms is lowest. This is shortly after micturition when the effect of dilution by fresh urine is maximum. O'Grady and Pennington (1966) showed that if diluted medium was added to a model bladder this enhanced the effect of the dilution, and conversely if the urine was already infected it detracted from the effect of dilution.

Computer models

O'Grady and his associates (O'Grady et al., 1968) have taken this work a step further by programming first an analogue computer and then a digital computer with mathematical data in order to predict more accurately the fate of bacterial population in the bladder under different hydro-kinetic conditions. The authors are satisfied that their models, both mechanical and computer, are sufficiently in accord with the in-vivo response to enable them to proceed to the detailed study of the effects of the dilution of urine that occurs in forced diuresis and of antibacterial therapy. They observe that "the most exciting single prospect of these models is that they may make it possible to unravel the extra-ordinarily complicated interactions which occur between numerous factors in the infected urinary tract to the extent that the dominating influence in any particular conditions may be identified, and subsequently manipulated to obtain the best possible outcome of therapy".

Antibacterial therapy in in-vitro models

O'Grady and Pennington (1967) demonstrated how ampicillin added to a fully grown culture of Proteus mirabilis (which was sensitive to 3 µg per ml by conventional sensitivity testing) to yield a concentration of 1000 µg per ml only marginally reduced the rate of growth of the culture in the model bladder as further medium was added to the bladder. If, however, the fully grown culture was first diluted by the addition of 'ureteric urine' at a rate of 1 ml per minute for two hours, and then the bladder voided leaving 30 ml of residual urine and the ampicillin added at this point to a concentration of 80 - 100 µg per ml, and a second dose of ampicillin administered after a second voiding 2 hours later complete inhibition of

growth occurred.

It should be noted at this stage that the ampicillin was not added pharmacologically. The total amount was given at one instant, and it was diluted along with the urine and the infecting organism. Secondly the measurements of bacterial numbers were made with a nephelometer, and were not viable counts. However, the authors demonstrate the very great difference in the amount of ampicillin requiredto inhibit the growth of the organism in the diluted culture compared with the fully grown culture, and they suggest that the resistance of the fully grown culture may account in a substantial part for the resistance to treatment of patients whose urinary tract harbours large volumes of infected urine.

Variable response by different organisms in the in-vitro bladder

In 1969 Greenwood and O'Grady reported unexpected differences in the react-:ion of different organisms to antibacterial therapy in the in-vitro bladder. They used 12 strains of E. coli sensitive to 2 - 16 µg ampicillin per ml, and 14 strains of Proteus mirabilis sensitive to 1 - 4 µg ampicillin per ml. The strains of Proteus included 5 non-swarming strains. An overnight broth culture of the test organism was diluted 1 in 4 and ampicillin was added to give a concentration of 66.6 µg per ml at the beginning of the experiment, and this solution was then subjected to a 1 ml per minute dilution in the model bladder. The opacity of the cultures of E. coli dropped very quickly, faster than could be accounted for by inhibition, indicating lysis of the organisms, whereas the strains of Proteus were more slowly affected. There was also a difference between the swarming and non-swarming strains of Proteus. The opacity of the swarming strains of Proteus decreased most slowly, the non-swarming strains

forming an intermediate group between strains of E. coli and swarming Proteus.

The different reactions were correlated with the number of persisters recovered from one ml samples of broth taken from tubes showing inhibition of growth in the conventional tube-dilution test. The strains of E. coli showed little survival above the mean inhibitory concentration, but the strains of Proteus mirabilis fell into two groups, the swarming strains showing large numbers of survivors even in high concentrations of ampicillin, whilst the non-swarming strains were intermediate between the two groups. Electron-microscopic evidence was also presented showing differences in the susceptibility to ampicillin of individual cells within the bacterial population.

Hypertonicity of medullary urine

Andriole and Epstein (1965) demonstrated the protection that water diuresis conferred on experimental rats. Experimental and control rats were each given an intravenous injection of staphylococci or Candida albicans. The experimental rats were protected by a water diuresis induced by adding 5% glucose to their drinking water. Only 3 of the 17 experimental rats succumbed to a urinary infection, while 16 of the 17 control rats did so. Moreover, none of the experimental rats had renal abcesses which were a feature of the control infections.

Following the intravenous injection of bacteria the number of organisms lodged in the liver and spleen of experimental and control rats were assayed, and no difference was found.

The authors consider that the principal factor involved was a reduction in the tonicity of the medullary urine, and they review the reasons why this might be so, and in the general discussion they consider the factors that

cause urinary infections to be located in the medulla rather than the cortex of the kidney. It is known that hypertonic saline, and concentrations of saline and urea within the range present in the urine, inhibit the phagocytosis of E. coli by leucocytes (Hamburger, 1912; Chernew and Braude, 1962), and the activity of complement (Kabat and Mayer, 1948). Moreover since a water diuresis increases meduallary blood flow (Thurau, Deetjen and Kramer, 1960) more of the blood-borne defence factors such as phagocytes, antibody, lysozymes etc., would be delivered to the kidney of the experimental rats. Lastly they comment on the fact that protoplasts that might be formed during antibacterial therapy, or by the action of antibody or complement, are preserved in a hypertonic environment, but lysed in dilute urine (Braude, Siemienski and Jacobs, 1961).

Other factors that might contribute to the susceptibility of the medulla compared with the cortex, to urinary infection were the anatomical position of the former, its relatively poor blood supply, and its content of ammonia producing enzymes.

LABORATORY DIAGNOSIS OF URINARY TRACT INFECTION

Bacteriuria means that the voided urine contains bacteria, it does not specify the origin of the bacteria, which may be the bladder urine, or it may be the urethra or peri-urethral skin. To distinguish between these possibilities Kass (1956) introduced the statistical concept of 'significant bacteriuria'. Briefly, in a mid-stream specimen of urine that has been properly collected, contaminants are unlikely to be found in greater numbers than 10,000 per ml. and organisms that are multiplying in the bladder urine are unlikely to occur in smaller numbers than 100,000 per ml. Clearly, since it is a statistical concept, urines may contain bacteria in excess of 10,000 per ml, or even 100,000 per ml as a result of contamination (false-positive diagnosis), and urines may contain less than 100,000 - and less than 10,000 - bacteria per ml as a result of infection (false-negative diagnosis).

False-negative diagnosis

A false-negative diagnosis is perhaps, more important than a false-positive diagnosis because a genuine case of urinary infection may be missed. Roberts, Robinson and Beard (1967) demonstrated how the viable count in the urine of untreated patients with urinary infection declined steadily during the day from the maximum in the first specimen of the day, and that in the presence of an increased fluid intake a count of only 10^3 organisms per ml was possible. Ambrose and Hill (1965) showed, with 146 specimens from 85 patients with proven urinary tract infection showing radiological changes, who were not receiving anti-microbial therapy, that 35 per cent. of the specimens were 'No growth', and a further 30 per cent. had fewer than 10,000 bacteria per ml. Thirty-three per cent. of patients

investigated by Stamey and his colleagues (1965) who had urinary infection had a viable count of less than 100,000 bacteria per ml. Among the reasons for low viable counts in urinary tract infection are an extreme diuresis, a highly concentrated acid urine (Asscher, et al., 1966) and the presence of antibiotics in the urine, but in most cases there is no obvious cause.

False-positive diagnosis

If a false-negative diagnosis is the more serious error, a false-positive diagnosis is probably the more common. Stamey et al. (1965) found that of 54 female patients with a sterile urine* obtained by suprapubic aspiration, only one patient voided a sterile urine when a mid-stream urine was examined. Including this patient 81.5 per cent. of the patients had viable counts of less than 10,000 bacteria per ml, 11.1 per cent. had counts between 10,000 and 100,000 and 7.4 per cent. had viable counts in excess of 100,000 organisms per ml. This appears to be a problem nearly confined to the female sex, because among male patients with a sterile suprapubic specimen 64 per cent. provided sterile mid-stream urines.

Peri-urethral cleansing

Elaborate preparations to cleanse the peri-urethral area have been advocated for women, but Turner (1961) compared 200 prepared women with 200 who had had no cleansing and found no significant difference in the incidence of contaminated urines, and Sleigh, Robertson and Isdale (1964) confirmed this. Roberts et al. (1967) found that swabbing the vulvae with chlorhexidine significantly reduced the viable count of the mid-stream urine compared with the count of the supra-pubic specimen taken at the same time, but Braude et al. (1967) found that washing the perineum of infants (without antiseptic) caused

* A sterile urine was one containing fewer than one organism per ml.

an increase in the number of bacteria per ml isolated from 'bag' urines.

Much, however, depends upon the patient. Most of the work in this field has been carried out on active, generally healthy and co-operative young women in an early stage of pregnancy. It is not reasonable to apply the conclusions obtained with such a group to infants or geriatric or unco-operative patients, or to those who have recently had gynaecological or urological operations. In an annotation in the Lancet (1962) the difficulties associated with the collection of mid-stream specimen of urine in a busy outpatient clinic are exposed: "In women patients a mid-stream specimen collected with care under expert guidance serves admirably; but not so the specimen obtained after brief and hasty instruction. Picture the elderly patient, clad for inclement weather in heavy coat, scarf, hat and gloves, and carrying a 1 oz bottle, being directed to a toilet box with a few half remembered words of instruction going round her head. How can a suitable specimen be expected".

Collection of specimens

There does not seem to be any alternative when making a diagnosis to a consideration of the results of three or more carefully collected specimens of urine in the light of the clinical situation. Certainly no rule of thumb is acceptable if the object is an accurate diagnosis.

The method of the collection of urine specimens has received some attention in recent years. The use of the catheter has been largely discredited as a diagnostic procedure since the risk of infection following a single catheterisation by expert hands is estimated to be between 2 and 6 per cent. (Marple, 1941; Kass, 1956; Brumfitt, Davies and Rosser, 1961). Kass gave up using catheters in favour of "clean voided" specimens obtained after washing the perineum with soap and water and voiding into a sterile beaker. The precise

method of obtaining 'mid-stream specimens of urine' or 'clean catch' specimens is rarely detailed, and yet it is of some importance. Whether or not there has been any interruption of the stream of urine when taking a mid-stream specimen is rarely recorded, and yet Stamey (1965) has shown that urethral organisms can reaccumulate in the urethra very quickly, and the stranguary which often accompanies interruption of theflow might result in prostatic or other glandular secretions along with any bacteria they may harbour, being injected into the remaining bladder urine. The technique used by Vejlsgaard (1965), Stamey et al. (1965) and Roberts et al. (1967) by which a tube or bottle is introduced into the un-interrupted flow of urine eliminates these sources of error and the pain that may be associated with stopping the flow at the expense of a tiny spillage of urine.

In infants and young children the difficulties of obtaining a specimen are more acute. Adhesive plastic bags have been discredited. Newman et al. found that 64 per cent. of 162 thriving neonate boys and girls examined by this method had over 10^5 bacteria per ml of urine, whilst follow up examination indicated that none was infected. Cruickshank et al. (1967) however have demonstrated that with time and patience good quality mid-stream specimens can be obtained even from neonates. In their series only 14 per cent. of 120 specimens from neonates yielded a growth of more than 100,000 bacteria per ml.

In situations where doubt persists it would seem that supra-pubic aspiration of the bladder is less dangerous than catheterisation, and this procedure should be carried out both in adults and infants. Stamey et al. (1965) developed the technique of supra-pubic aspiration and proved its safety by performing over 2500 aspirations without a single complication,

and its acceptibility to the patient by performing the operation over 40 times on each of two patients and over ten times on many other patients. Newman, O'Neill and Parker (1967) demonstrated the safety of the technique with children, but it has not yet supplanted the catheter.

Viable counting of bacteria in specimens of urine

Accurate viable counts on a specimen of urine may be made either by pour plate techniques, or by the surface viable counting methods of Miles and Misra (Miles, Misra and Irwin, 1938). Unfortunately these methods are costly in time and materials. Many routine bacteriological laboratories are experiencing an increase in the number of requests for examinations of 10 - 15 per cent. per year (J. Clin. Path., 1968) and a considerable proportion of this increase is in requests for examination of specimens of urine. Many of these come now from antenatal and other clinics and are screening tests for asymptomatic bacteriuria, and of course most of them are negative. There is obviously, a demand for less expensive methods sacrificing if necessary some accuracy.

The second problem which has been encountered is the demand for a method of counting the bacteria present at voiding in samples submitted to the laboratory after a considerable delay during which small numbers of contaminating organisms may have multiplied and assumed 'significant' proportions. A variety of techniques have been developed to meet these situations.

Laboratory screening tests

The calibrated loop technique of Cattell and Leeford (1963), modifications of which are widely used, involves spreading a 2 mm loopful of urine over the whole surface of an agar plate, and counting the colonies after incubation.

Good correlation can be obtained between this method and more accurate counting techniques. McGeachie and Kennedy (1963) used a slightly different method. A standard loopful of urine was inoculated along one edge of a box forming a 'well', and then with further sterile loops in turn this inoculum was spread out along the other three sides of the box. Again a good correlation was obtained when the method was compared with more expensive techniques.

The blotting paper strip method suggested by Ryan, Hoody and Luby (1962) and developed by Leigh and Williams (1964) involves the use of blotting paper strips of a standard porosity with a folded tip of a standard size. The sterilised strip is dipped into the specimen of urine to immerse the tip, and then after allowing the excess fluid to be absorbed the tip is laid onto the surface of a well dried MacConkey plate. The number of colonies are compared with numbers obtained in calibration experiments. Good correlation with accurate methods is possible, but the preparation of the filter paper strips is tedious.

It must be emphasized that these are screening tests. Urquhart and Gould (1965) who developed a further modification of the calibrated loop technique, commented that a degree of accuracy of one log, i.e. 10 fold, was enough under these circumstances. Where counts fell into the critical urinary range of 10^4 to 10^5 bacteria per ml, they considered that several specimens should always be assessed, and the clinical circumstances taken into account.

Viable counting techniques suitable for use in general practice

The 'semi-quantitative' counting methods described above were devised principally for laboratory convenience in order to give a useful result without an increase in the work load of the laboratory. Three more recent screening

techniques however, have eliminated the difficulties experienced by general practitioners and small hospitals remote from a bacteriological laboratory where it has been impossible to examine a specimen soon after it has been passed, and difficult to refrigerate it in transit to the laboratory.

(1) The dip inoculum spoon of Mackey and Sandys

The dip inoculum spoon (Mackey and Sandys, 1965) consists of a screw capped bottle containing a small plastic spoon filled with agar. A spike protruding from the bowl of the spoon pierces the cotton wool pad in the bottom of the bottle and achors the spoon in transit. A non-inhibitory medium, the cysteine lactose electrolyte deficient medium (CLED medium) of Sandys (1960) is used in the spoon. Urine is passed into another sterile container, the spoon is dipped into it, replaced into its own container and posted to the laboratory. After incubation the colonies are identified and counted and a quantitative estimate of the bacteriuria can be made. It was found in calibration experiments that with the increase in the viable count the number of colonies per spoon increased, and their size diminished so that a straight line relationship was maintained up to near the point where the colonies coalesced, (see Figure 1).

Fewer colonies of Gram-positive cocci developed on the spoon compared with colonies of Gram-negative bacilli for the same number of bacteria per ml, the explanation offered being the break up of chains and clumps of cocci on spreading the plates in the control experiments.

Approximately 20 or approximately 16 colonies represented 10^4 Gram-negative bacilli or Gram-positive cocci respectively and 200 or 160 colonies represented 10^5 of the respective bacteria per ml. Up to about 250 discrete colonies could be counted before they began to coalesce. Counting of the

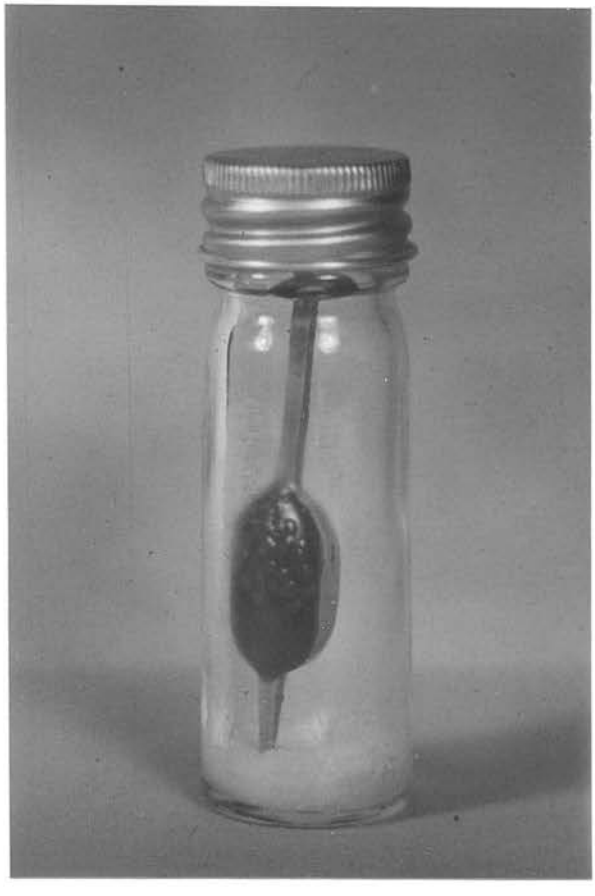
FIGURE 1

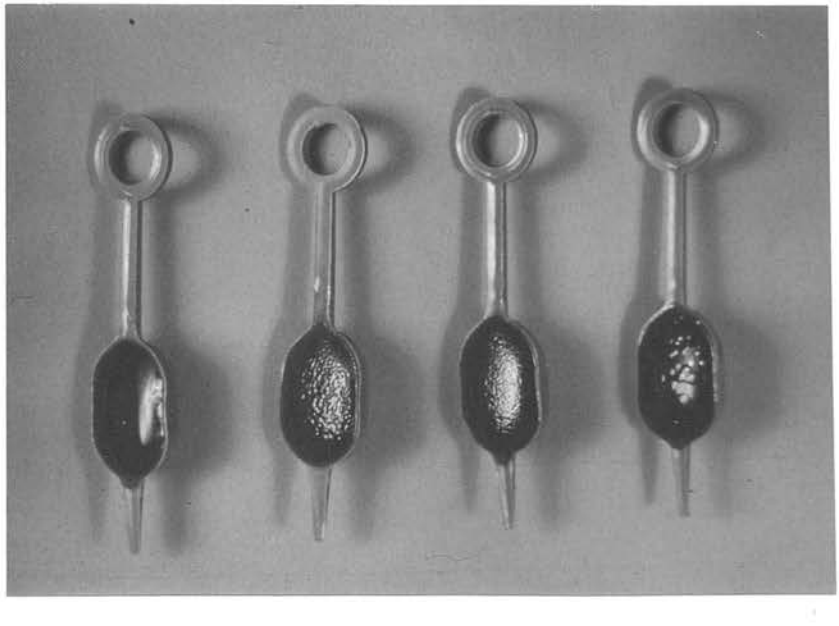
The dip- or stream-inoculum spoon

The upper picture illustrates an outfit after overnight incubation. This spoon had 50 lactose fermenting colonies upon it, representing between 10^4 and 10^5 bacteria per ml of urine. The spike of the spoon impales a cotton wool pad. In later outfits a polyurethane foam wad replaced the pad. Magnification x 1.

The lower picture illustrates four spoons after inoculation and overnight incubation. The first one (on the extreme left) shows 'No growth'; the next one has about 150 colonies, the third spoon has a confluent growth and the fourth spoon is the same one that has been illustrated above.

The pale disk in the agar that is visible in some of the spoons is a stud which anchors the agar and prevents it from falling out during transit. Magnification x 0.75.





colonies on the spoons from most patients was not required because there were either no colonies at all, or very few, or because the growth was confluent.

The advantages of the system are: (1) time spent in the post does not affect the results, (2) antibiotics that may be present in the urine are less likely to mask a positive result, and (3) the system is economical of technicians' time as no plating of specimens is required and only positive specimens are examined further. The obvious disadvantages are: (1) that the urine deposit cannot be examined, (2) where there is no growth it is impossible to tell whether the specimen has been inoculated, and (3) the complete outfit is quite expensive.

Mackey and Sandys work was with a varnished metal spoon, but a plastic spoon of similar proportions became available in 1966 (Mackey and Sandys, 1966).

The dip inoculum spoon method has been used extensively and copied widely. The most notable modification has been by Guttman and Naylor (1967) who developed the dip slide. This is a microscope slide (7.5 by 2.5 cm) coated for 5 cm with nutrient agar on one side and MacConkey agar on the other. It has the advantage of holding two media upon which growth can be compared, and it has a much larger surface area. The increase in the area however is of questionable value as the density of colonies is not different from that recorded by the spoon, and the greater size means that more urine is required to cover the slide and there are more colonies to count. Moreover Wright (1968) found that if freshly prepared the agar tended to slip off the slide, and if old it tended to dry up. Commercial modifications of the dip slide are available from several companies and promise to be useful tools for the diagnosis of urinary infection by

general practitioners.

(2) Boric acid urine preservative

Porter and Brodie (1969) have shown that a solution of 1.8 per cent. boric acid in urine preserves the red and white cells, and prevents the bacteria from multiplying. A measured quantity of boric acid is added to the container in the laboratory before the container is issued to the practitioner. The advantages of the system are: (1) its economy of preparation, (2) the availability of the urine deposit for examination, a factor that may not be of much value but which is requested and appreciated by the practitioners, (3) the fact that no new techniques have to be learned by patients or laboratory staff.

The difficulties reported were: (1) the bottle had to be filled to the brim (28 ml) in order to get the right concentration, and small children would find it difficult or impossible to pass this amount especially as a mid stream urine, (2) some patients tipped out the powder before filling the bottle, but this difficulty can be discovered if not surmounted by testing the urine for boric acid in the laboratory, and (3) that a few strains of proteus were found to be sensitive to boric acid and to suffer from a reduction in numbers during transit.

(3) Modified blotting paper strip method

The 'Testuria' outfit (a commercial production) consists of a strip of blotting paper similar to that used by Leigh and Williams (1964) which is dipped into the urine and then laid momentarily on to a small agar plate. A cover is clipped over the plate forming an airtight seal and it is then posted to the laboratory where it is incubated and evaluated.

Chemical tests

A third group of screening tests for bacteriuria are the chemical tests. These have the advantage that they can generally be read by unskilled personnel and usually they are cheap. The Ilosva modification of the Greiss test has been widely used, but unless excess nitrate is added and the urine incubated for four hours, which is inconvenient in most circumstances, a high number of false negatives are obtained, and even with this modification infections by Str. faecalis will be missed. The Triphenyl Tetrazolium Chloride (TTC) test is perhaps the best for use in general practice, but it has to be carefully standardised, and even so is likely to miss infections due to Ps. aeruginosa (which are, however, uncommon outside the hospital environment). A new test based on the fact that glucose is the main source of energy for the bacteria in the urine detects subnormal levels of urine glucose (Schersten et al., 1968), but the difficulties with this test include the fact that the patient must fast overnight before the specimen is collected, and it needs further evaluation.

NATURE OF PENICILLIN RESISTANCE

Fleming in 1929 noted that E. coli and other Gram-negative bacilli were not inhibited by penicillin. Abraham and Chain (1940) confirmed this and considered that it was due to an enzyme which they called penicillinase. They made an extract of E. coli by crushing the bacilli, and they found that it had an inactivating effect on penicillin. They postulated that it was an enzyme because:-

(1) it was destroyed by heating at 90° C for 5 minutes,

(2) it was destroyed by papain activated by potassium cyanide, and

(3) it was not dialysable through cellophane membranes.

They noted that its activity was slight at pH 5, but that it was very active at pH 8 and pH 9.

Bondie and Dietz (1946) showed that penicillinase producing organisms were most common among the coliform group and the Gram-positive spore bearing bacilli, and they demonstrated that the enzymes produced by different organisms varied, differing for example, in their stability to heat. Abraham and Chain (1940) noted that some strains of Gram-negative bacilli produced penicillinase that was liberated into the medium, whereas in others it was all cell bound. Eagle (1954a) confirmed this and noted that the cell-bound enzyme could be liberated by ultra-sonic waves. Collins (1964) found that the cell-bound penicillinase consisted mainly of the more recently synthesised enzyme molecules.

Kinetics of penicillinase activity

In 1948 Gilson and Parker working with staphylococcal penicillinase found that the destruction of penicillin proceeds regularly with time, and that it was proportional to the temperature within limits, and that the rate

of destruction of penicillin was proportional to the concentration of penicillinase. They established that the rate of penicillinase production by a strain of staphylococcus was constant over five generations.

Sutherland (1964) demonstrated that, with an intrinsically sensitive, penicillinase producing organism, growth in a culture to which penicillin had been added was inhibited until the penicillin had been reduced to a sufficiently low level. In the experiment reported the strain of E. coli that was sensitive to 25 µg of penicillin per ml would not grow until the penicillin concentration had been reduced to 3.0 µg per ml. However, the work of Bondie and Dietz (1948) indicates that the situation is more like a race between the inactivation of the penicillin by the enzyme, and the destruction of the organisms by the penicillin. Bondie found that the susceptibility of a penicillinase producing organism to penicillin depends on its ability to produce penicillinase quickly. Bacillus megatherium, for example, if incubated for 24 hours or longer will produce large quantities of penicillinase, but an inoculum of 10^3 organisms will produce very little in the first four hours, and the organism is sensitive to penicillin. Bacillus cereus, on the other hand, although it will not produce as much penicillinase over 24 hours, will produce copious quantities of the enzyme from an inoculum of 10^3 organisms in the first four hours of incubation, and it is relatively resistant to penicillin.

Proportional relation of penicillinase production and penicillin resistance

Percival, Burmfitt and de Louvois working in 1962 with Gram-negative bacilli produced results which conflict with those of Bondie and Dietz (1948). They found a proportional relation between ampicillin and penicillin G

resistance and the amount of penicillinase produced in three 'trained' penicillin resistant strains, and in several (but not all) naturally resistant strains. They showed that trained penicillinase-producing penicillin-resistant variants of penicillin-sensitive strains were no more resistant to the penicillin 'BRL 1621' than were the parent strains, this compound being resistant to penicillinase. However penicillinase production by these organisms was small compared with the amounts produced by Gram-positive organisms such as penicillin resistant staphylococci.

Types of enzyme inactivating penicillin

Coliform bacilli may produce either, or both, or neither, of two enzymes that inactivate penicillin. Beta-lactamase is the enzyme that splits the beta-lactam ring, and the term penicillinase has been, and will be reserved in this thesis for this enzyme alone. When penicillinase acts on penicillin G or ampicillin, penicilloic acids are produced. The acids resulting from the breakdown of ampicillin and penicillin G differ, but since both reduce iodine to the same extent, which is the basis of the quantitative estimation of penicillinase activity (Ayliffe, 1963), the difference is not important.

The other enzyme is an amidase which splits off the side chain leaving the penicillin nucleus intact. Though commercially a most useful enzyme, it is much less important than penicillinase clinically. Ayliffe (1963) found no evidence of penicillin amidase production in 148 strains of coliform bacilli but some degree of amidase production could not be excluded. Sutherland (1964) examined an unspecified number of strains of E. coli, Proteus Ps. aeruginosa and Aerobacter aerogoenes in which there was a marked destruction of penicillin, and found evidence of the production of

penicillin amidase in appreciable quantities in only one strain of Proteus rettgeri. Even in this strain penicillinase was also produced.

Non-penicillinase mediated penicillin resistance

Penicillinase production is not the only method by which bacteria resist the lethal action of penicillin. Non-penicillinase producing Gram-negative bacilli are 1000 times more resistant to penicillin than non-penicillinase producing staphylococci. Furthermore, it was shown with staphylococci by Demerec in 1945 that it was possible to produce a gradual increase in resistance to penicillin by growing the organisms in vitro in increasing concentrations, and Bondie and Dietz (1946) demonstrated that this resistance was not due to penicillinase production. Rolinson and Stevens (1961) noted a similar stepwise increase in penicillin resistance with a strain of E. coli but no estimation of penicillinase production was made.

Klimek, Cavillito and Bailey (1948) managed to increase the resistance of a strain of staphylococcus from 0.1 Oxford unit (0.06 µg) of penicillin per ml to over 1000 units (600 µg) by repeated sub-culturing in increasing amounts of penicillin. Initially before very high levels of penicillin resistance were achieved, the organisms reverted rapidly to penicillin sensitivity on returning to a penicillin-free medium. But eventually there was a change in the morphology of the organism, and all the characteristic biochemical reactions were suppressed, growth was slower, and in spite of the fact that no penicillinase was produced the organism did not revert to penicillin sensitivity when penicillin was removed from the culture medium.

Three different types of penicillin resistance

Eagle (1954a) considered that there were three different types of penicillin resistance. The most important was the production of penicillinase

which was either liberated into the medium, or remained cell bound. Secondly he postulated that some species of bacteria had a 'low reactivity' to penicillin. He found (Eagle, 1954b) that the amount of penicillin bound to an organism at a low (non-lethal) concentration of penicillin was inversely proportional to the sensitivity of the organism to penicillin. Thus a strain of Streptococcus pyogenes might have 400 times as much penicillin bound to its cell wall as a strain of E. coli. However, the amounts bound to the surface of cells of different species of bacteria were similar in all cases if the penicillin concentration was at the minimum inhibitory concentration for each. The hypothesis is that the difference in penicillin sensitivity is due to the varying reactivity of cell components with penicillin.

The least important mechanism postulated by Eagle (1954a) for mediating penicillin resistance was that by which strains of staphylococci develop resistance without penicillinase production. In these there was no alteration in the quantities of penicillin bound to the cell wall. This mechanism has not been explained.

Small amounts of penicillinase confer a high degree of penicillin resistance

Percival et al. (1962) found that the penicillin and ampicillin resistance of several naturally resistant Gram-negative bacilli was proportional to the amount of penicillinase produced, but that this was small compared with the amounts produced by Gram-positive organisms. The reason for this discrepancy is not clear but they believed that it might be related to the presence of the lipid in the Gram-negative cell wall.

Production of penicillinase producing variants from non-penicillinase producing strains

The production of a penicillinase-producing variant of a non-penicillinase-

producing staphylococcus has been the subject of many experiments. Roy and Kankford (1954) and Szybalski (1953) developed different methods for the isolation of a single penicillinase-producing cell from 10^6 to 10^8 non-penicillinase-producing cells. In 1962, however, Percival et al. managed to obtain penicillinase-producing variants of non-penicillinase producing strains of E. coli without difficulty, but they were able to demonstrate minute amounts of penicillinase in one of the parent strains. Smith (1963), however, isolated a penicillinase-producing variant of a strain of E. coli by accident, but found it impossible to repeat this fortuitous event, and considered that it was a rare mutation.

Loss of the ability to produce penicillinase

Whereas it is very difficult to obtain a penicillinase-producing staphylococcus from a sensitive strain Barber (1949) demonstrated that the reverse change was common. Using ditch plates and cultures kept on the bench for several months she demonstrated that a penicillinase-producing strain throws off penicillin sensitive variants relatively frequently. In the absence of the selecting influence of penicillin therefore there would appear to be a tendancy for penicillin-resistant staphylococci to become penicillin-sensitive.

There are few references in the literature to cases of urinary tract infection with Gram-negative bacilli that have been successfully (or unsuccessfully) treated with oral penicillin G. Before penicillin became cheap and plentiful it was wasteful to give it orally, and certainly in the early days when the supply was very limited, and sulphonamides were available, it could be more profitably employed against staphylococcal and other Gram-positive infections. Furthermore, it was clear that without massive parenteral doses the level of penicillin in the blood was unlikely ever to approach the mean inhibitory concentration of most of the Gram-negative bacilli.

Scowen, Badenoch and Shooter (1957) give a very full report on a patient who was successfully treated with oral penicillin G. The child was first seen aged $4\frac{1}{2}$ when he was still enuretic. Investigations revealed that he had bilateral calculi, and, by the time he was eight years old, both had been removed but had recurred. Culture of his urine grew E. coli and Staph. aureus on one occasion and E. coli and Staph. albus on another. Both stones were removed a second time, but within a month there was radiological evidence of recurrence once more.

Chemotherapy post-operatively with tetracycline eliminated the staphylococcus, but left a coliform bacillus. Laboratory studies indicated that penicillin was bactericidal at 50 units (30 µg) per ml, and that a combination of penicillin and streptomycin (the latter at a 100 µg per ml) was also bactericidal, whereas combinations with chloramphenicol or tetracycline were bacteristatic. Treatment was therefore started with penicillin 12 mega-units (7.2 g) daily parenterally, and streptomycin 0.5 g daily for seven days. This sterilised

the urine, but infection recurred three days after the regime stopped. Oral penicillin and mandelic acid was then tried, but the latter was not well tolerated, and so penicillin was given alone. A daily dose of 4,200,000 units (2.525 g) of benzathine penicillin G was given for six months. From the second day of treatment the urine was sterile, and it remained so until the child was last seen three years after the regime had begun. Pus was present in the urine at first, but the amount gradually decreased, and disappeared. This is the only patient given oral penicillin therapy for Gram-negative urinary infection which the author has seen reported in detail prior to 1965.

Peeney (1947) reports the successful treatment of two patients with Gram-negative urinary tract infections with penicillin G. In the first a strain of E. coli sensitive to 83 units (50 µg) of penicillin per ml was eliminated from the urine in 5 days by a continuous intramuscular drip of penicillin at a rate of 400,000 units (250 mg) per day. A five to one oral/parenteral ratio makes this parenteral dose equivalent to 2,000,000 units, or 1.2 g per day by mouth. The second patient suffered from a cystitis caused by Proteus morganii that was cured by twice daily instillations into the bladder of 100 ml of saline containing 200 units (120 µg) of penicillin. The organism was sensitive to 50 units (30 µg) per ml.

Stamey, Govan and Palmer (1965) give an account of 15 patients who were treated with oral nitrofurantoin or penicillin. Neither of these drugs achieves bactericidal concentrations in the serum, and Stamey and his colleagues were endeavouring to demonstrate that urine concentrations of the antibiotic were sufficient to cure proven cases of pyelonephritis as

opposed to cases of 'cystitis' or 'pyelitis'.

Radiological studies were carried out on all, and the ureters were catheterised in 8 cases, and the ureteral urine collected and cultured. In the other seven cases the pre-treatment specimen was obtained by supra-pubic bladder aspiration. Most specimens taken during and after treatment were obtained by supra-pubic aspiration, but some were mid-stream urines. Seven patients had radiological abnormalities and all had significant growths of Gram-negative bacilli in the pre-treatment specimens of urine.

Four patients were treated with penicillin G 400,000 units (250 mg) orally four times daily, eight were given 1 g of penicillin V daily in four doses, and three were treated with nitrofurantoin, 100 mg orally four times daily. All drugs were given for 10 days. The patients were followed up for periods varying from 3 days to 6 months. In no case was a significant number of bacteria cultured during treatment, and in all but two cases the urine remained sterile during the follow-up.

Of the two patients who failed treatment one was treated with nitrofurantoin and was shown to have a stone in the right kidney. Her urine was sterile 21 days after treatment, but infected again by the sixth week. The other patient (treated with penicillin G) was later shown to have recurrent infections associated with a large residual volume and sexual intercourse.

Types of treatment failure

Stamey and his colleagues found that when an antibiotic is effective the urine becomes sterile within 48 -72 hours, and is probably sterile within 24 hours in many cases although a few have less than 100 bacteria left in the bladder on the fourth day. When an antibiotic failed to cure Stamey found

that he could place the case into one of three groups.

(1) Infecting organism replaced by pre-existing resistant organism

In this, the largest, group the organism was eliminated but replaced by another organism of a different genus. This could not be a mutation, and occurred too quickly to be a re-infection, and Stamey concludes that in these cases both organisms were probably present initially, but that the greater numbers of one obscured the other, which was resistant to the drug used. To these patients Stamey gave additional treatment while continuing the antibiotic for the first organism.

(2) Persistence of infecting organism

In this group the original organism persisted with an unchanged sensitivity. Stamey could usually find a reason for this persistence in the face of a bactericidal antibiotic, e.g. a very large residual urine which diluted the antibiotic, or a hydronephrosis, or a severe uraemia with greatly deminished excretion of penicillin. The placing of a case in this group should alert the clinician's attention to a cause for the failure.

(3) Altered antibiogram of infecting organism

The smallest group was drawn entirely from those patients who had been treated with penicillin. In these cases the infecting organism which had been sensitive to penicillin became resistant to it. When treatment was altered to account for this the patients were cured.

Suppression and re-infection

Finally Stamey found that in patients with calculi or other severe renal abnormalities although the urine was sterilised during treatment the infection recurred with the original organism within 48 - 72 hours after

stopping the antibiotic, because the bacteria were not completely eradicated. Thus he points out that suppression of infection results in a recurrence of bacteriuria within a matter of hours rather than days after cessation of treatment.

SECTION 1

Relation of penicillin sensitivity of Gram negative bacilli to the concentration of penicillin attainable in the urine with oral therapy

INTRODUCTION

In the experiments recorded in this section an attempt is made to establish the theoretical basis for the treatment of urinary infection with oral penicillin G.

The concentration of penicillin in the urine was measured in a few random subjects who were taking penicillin G, and then a cross-over trial of three preparations of the drug was carried out with six young volunteers.

The penicillin sensitivities of a large number of Gram-negative bacilli were measured both by the tube-dilution method, and by the use of a filter-paper disk impregnated with penicillin. As no commercial disk existed for this purpose a disk was specially developed and produced.

Some investigations were made into the nature of penicillin resistance of Gram-negative bacilli involving the production of penicillinase, and some of the classic experiments performed originally on staphylococci were repeated with strains of E. coli.

Finally an in-vitro model bladder was set up to test the use of penicillin in conditions simulating as closely as possible those that exist in vivo.

MATERIALS AND METHODS

Media

'Oxoid' MacConkey Agar, 'Oxoid' Nutrient Agar, and 'Oxoid' Blood Agar Base No. 1, with 5 per cent. by volume of horse blood were used for plate cultures. 'Oxoid' Diagnostic Sensitivity Test Agar Base (without the addition of blood) was used for all disk-diffusion tests. 'Oxoid' Nutrient Broth was used for all broth cultures and the tube-dilution sensitivities. Dorset's egg slopes (Cruickshank, 1969 page 756) were used to store cultures. 'Oxoid' Koser Citrate medium was used to test the ability of Gram-negative bacilli to utilise sodium citrate as the sole carbon source, and 'Oxoid' Urea Broth Base to detect urease production. Media to test various carbohydrate fermentation reactions and other biochemical tests were prepared as detailed in Medical Microbiology (Cruickshank, 1969 page 747).

Subjects

Six healthy subjects volunteered to take part in the experiment. Five were male and one was female. The age range was 17 - 28 years, and the weight range was 60 - 76 Kg. No instructions about food or fluid intake were given save that the drug should be taken 30 minutes before breakfast immediately after the bladder had been emptied. At least one day elapsed between tests of different preparations of penicillin in the same volunteer.

Penicillins

Potassium penicillin G. (Benzyl-penicillin). 'Crystapen G' (Glaxo Laboratories, Ltd) is presented in 250 mg sugar coated tablets, and the dose used was two tablets. (Since this trial was carried out the sugar coated tablets have been replaced by film coated tablets). For tube-dilution sensitivity testing and for the production of penicillin filter-paper disks the same

preparation dispensed in 500,000 or 1,000,000 unit (0.3 g or 0.6 g) vials was used.

Sustained action penicillin G 'Hyasorb' (Berk Pharmaceuticals Ltd) is described by the makers as an acid-resisting formulation of penicillin G incorporating pellets that are specifically coated to disintegrate in the duodenum and small intestine in a timed release sequence that will maintain therapeutic serum levels (for Gram-positive bacteria) for up to 8 hours after one dose. Presentation is in 150 mg tablets and the dose used was three tablets.

Penamecillin 'Havapen' (John Wyeth and Brother Ltd) is an acetoxymethyl ester of penicillin G which is hydrolysed to penicillin G in the course of absorption from the gastro-intestinal tract. The makers claim that it is better absorbed than penicillin. The dose used was two tablets of 350 mg, each containing an equivalent of 305 mg of penicillin G.

The cost in the United Kingdom of one dose of each of the drug was: 'Crystapen G' 2p; 'Hyasorb' $6\frac{3}{4}$p; 'Havapen' 4p.

Specimens of blood and urine

In the controlled trial of penicillin excretion in the urine venous blood was withdrawn 2 and 6 hours, after the drug was given. The bladder was emptied at 2, 4, 6, 8, 12 and 24 hours and the whole of the specimen was collected. All specimens were dealt with within two hours of collection.

The specimens of blood were allowed to clot and then stored overnight at $4^{\circ}C$ to promote clot retraction. The serum was harvested with a pipette, centrifuged to remove remaining suspended red blood cells and filtered. The volume of each specimen of urine was measured, it was thoroughly mixed and an aliquot removed and filtered.

Filters

Twenty five mm diameter 'Millipore' filters with 0.45 μm pores were used for all filtrations. The filters are held in a patent attachment which fits on to the end of a syringe (see figure 2), and they are sterilised already assembled in an autoclave at 115°C for 10 minutes. These filters are ideal for sterilising small quantities of material because they do not absorb an appreciable quantity of fluid, nor do they alter its pH. They were tested to see if they absorbed any penicillin during filtration. Dilutions of penicillin containing 50 and 100 μg of penicillin G per ml of nutrient broth were made up. Using fresh filters on each occasion 6 small aliquots (2 - 3 ml each) and 6 larger aliquots (10 - 20 ml each) were filtered, and these were compared with the unfiltered solutions. No difference in the penicillin levels of the filtered and un-filtered solutions could be detected.

Measurement of penicillin concentrations in blood and urine

Ten doubling dilutions of the serum were made with nutrient broth. The urine was diluted 100-fold with nutrient broth and eleven doubling dilutions were made. Each tube (and a control tube of nutrient broth alone) was seeded with a single drop of a 1 in 100 dilution of an overnight growth in nutrient broth of the Oxford (Heatley) strain of Staphylococcus aureus which is inhibited by 0.02 μg of penicillin per ml but not by 0.01 μg per ml.

After incubation for 18 hours at 37°C the tubes were examined in a good light for turbidity. The end-point was the tube with the greatest dilution of serum or urine showing no turbidity. The level of penicillin was the dilution factor multiplied by 0.02. The range of penicillin concentrations detectable in the serum was 0.02 to 10.24 μg per ml, and in the urine 2 to 2048 μg per ml (Cruickshank, 1969 page 905).

FIGURE 2

Syringe and 'Millipore' filter holder

The filter holder is in two parts which may be unscrewed, and between which the filter is held. The holder and filter, already assembled, may be autoclaved.

Magnification x


5
10
ml
GMP

Controls

With each batch of measurments a control titration of the Oxford strain of Staphylococcus aureus was set up. On the few occasions when the measured sensitivity was not 0.02 µg of penicillin per ml, that batch of titrations was repeated. The sterility of each specimen following filtration was tested by incubating an undiluted aliquot of serum, and an aliquot of the urine which had been diluted 100-fold with nutrient broth. Filtration failed on two occasions.

With each batch of serum titrations a control titration of pooled normal serum to which penicillin had been added to a concentration of 1.0 µg per ml was included. No significant protein binding of the penicillin was demonstrated (Cruickshank, 1969 page 905).

Preliminary and subsequent experiments

Preliminary experiments were carried out with one of the subjects (JH) using potassium penicillin G. The conditions under which the experiments were carried out differed from those of the control experiment, and the results were sufficiently consistent and different from those of the main experiment to merit comment.

On three occasions hourly measurements of penicillin in urine over 6 hours were made after a dose of 500 mg of penicillin taken between 1 and 3 hours after breakfast. Facilities for filtration were undeveloped and so a mid-stream urine was collected to avoid contamination of the specimen, and it was not therefore possible to measure the volume of each specimen. However fluid intake was high (but unmeasured) in order to achieve hourly specimens and the urine output was also probably high, and the appearance was certainly of dilute urine.

A final preliminary experiment was performed under the conditions of the main experiment using a dose of 250 mg of potassium penicillin G.

Subsequently it was possible to measure the penicillin concentration in the urine of an adult undergoing penicillin therapy for urinary tract infection, and in the urine of a male child of $2\frac{1}{2}$ years (weight 14.5 Kg) who was receiving 125 mg of potassium penicillin G four times daily also for a urinary tract infection. In the latter case it was not possible to measure the volume of each specimen of urine, nor was it possible to get regular specimens. The drug was, however, given pre-prandially after the child had apparently emptied his bladder, and no urine was passed in the interval between the specimens which were collected for penicillin assay.

Bacteria

A total of 934 strains of Gram-negative bacilli and 35 strains of Gram-positive cocci were included in the investigations. The sources of these strains are set out in Table 3. Two hundred and forty three unselected but not consecutive isolates from hospitals in Aberdeen came from patients with urinary tract infections. Each strain was taken from a pure growth containing over 10^5 viable bacteria per ml of a mid-stream specimen of urine, estimated by the method of McGeachie and Kennedy (1963).

Forty eight consecutive strains were isolated from unselected patients with asymptomatic bacteriuria of pregnancy presenting at the antenatal clinic of the/

TABLE 3

Sources of the strains of bacteria investigated

Source	Number of strains of different species of bacteria							
	Total	*E. coli*	*Klebsiella*	Other Coliforms	*Proteus*	Other Gram-negative bacilli	Gram-positive cocci	
							Staph. aureus	*Str. faecalis*
Hospital	243	108	17	6	100	12*	-	-
General practitioner	502	305	26	20	57	60	4	30
Antenatal Clinic	48	38	0	0	3	6	1	0
Faeces from healthy adults	176	157	14	5	-	-	-	-
Total	969	608	57	31	160	78	5	30

* All 12 were strains of *Ps. aeruginosa*.

of the Aberdeen Maternity Hospital. These patients were not referred to the clinic for any disease or for any complication of pregnancy, but came for routine antenatal care. The 48 isolates came from 72 patients who had been recalled for a second examination following the discovery of asymptomatic bacteriuria on their first visit. Each isolate was a pure growth of more than 100,000 organisms per ml. of a mid-stream specimen of urine.

Five hundred and two strains from patients of family doctors in the City and County of Aberdeen were isolated consecutively. These were isolated in the course of the trial of penicillin G in the treatment of urinary tract

infection which is reported in Section III of this thesis. All isolates came from mid-stream specimens of urine containing more than 10,000 bacteria per ml, and the very great majority came from urines containing more than 100,000 bacteria per ml. Most, were pure.

One hundred and seventy six isolates were obtained from the faeces of healthy employees of a food manufacturing company. The subjects, both male and female, were undergoing routine screening for intestinal pathogens. The faeces were plated on to MacConkey medium and a representative colony of the predominant lactose fermenting strain was selected.

Identification of Gram-negative bacilli

Lactose-fermenting Gram-negative bacillary isolates in this survey were tested for growth in Koser's citrate, urease production and the production of indole in peptone water, after incubation at 37°C for 18 hours. Those strains that produced indole but did not grow in citrate or produce urease were identified as Escherichia coli. Those that did not produce indole, but grew in citrate were identified as Klebsiella regardless of the production of urease. Other lactose-fermenting isolates were termed "atpical coliforms".

Strains of Proteus were identified by their inability to ferment lactose and their ability to produce urease, and the different species were distinguished (when this was done) by production of indole in peptone water, growth in Koser's citrate and production of acid and gas from 1 per cent. maltose in peptone water. This identification scheme is illustrated in Table 4.

TABLE 4

Identification of bacteria

Organism	Tests				
	Production of indole	Production of urease	Growth in citrate	Acid and gas from maltose	Other observations
E. coli	+	-	-	NT	None
Klebsiella	-	+ -	+	NT	Colonial morphology
Proteus mirabilis	-	+	-	-	Swarming
Proteus vulgaris	+	+	-	+	Swarming
Proteus morganii	+	+	-	-	None
Proteus rettgeri	+	+	+	-	None
Ps. aeruginosa	NT	NT	NT	NT	Pigment production colonial morphology
Str. faecalis	NT	NT	NT	NT	Colonial morphology
Staph. aureus	NT	NT	NT	NT	Colonial morphology Coagulase test

NT - Not tested

The tests to differentiate the strains of Proteus were carried out on the isolates from the Aberdeen hospitals. Ninety seven were found to be Proteus mirabilis, three were Proteus vulgaris, and there were no strains of Proteus morganii or Proteus rettgeri. The differentiation of strains of Proteus was abandoned thereafter.

Identification of Gram-positive cocci

Gram-positive cocci were identified by colonial morphology, and in some cases by further tests. Staphylococci were tested with the slide coagulase test, and where this was equivocal by the tube coagulase test. Coagulase-negative strains of staphylococci were discarded. Recent work by Mabeck (1969) however, indicates that coagulase-negative staphylococci may cause urinary tract infection.

Where doubt existed about the identity of a strain a Gram film was prepared and examined and a colony was inoculated on to nutrient agar containing 10 g per cent. sodium chloride, and on to blood agar containing two parts per million crystal violet. The salt agar inhibited strains of steptococci but not staphylococci and the crystal violet agar inhibited strains of staphylococci but allowed strains of streptococci to grow.

Measurement of penicillin sensitivity

The penicillin sensitivity of strains of Gram-negative bacilli isolated from the Aberdeen hospitals was measured by tube-dilution (Cruickshank, 1969 page 901). A vial containing 0.3 g of crystalline penicillin G (occasionally vials containing 0.6 g were used) was dissolved in sterile nutrient broth and eight aliquots of broth containing, 2, 5, 10, 25, 50, 100, 150 and 300 µg of penicillin per ml were prepared. These aliquots were then distributed in racks of tubes in 2 ml amounts. The strains of Ps. aeruginosa and some strains of E. coli which were insensitive to 300 µg of penicillin per ml were tested in tubes containing 250, 500, 1000, 2,500, 5000 and 10,000 µg per ml. Fresh solutions of penicillin were prepared every day.

The inoculum was a single drop of a 1 in 100 dilution of a 6-hour-old broth culture, and it contained approximately 2×10^5 organisms. With each

batch of estimations the quantity of penicillin in the dilutions was verified by a control test using the Oxford (Heatley) strain of Staphylococcus aureus. If the mean inhibitory concentration (m.i.c.) of this test strain was not 0.02 µg of penicillin per ml the batch of sensitivity tests was repeated.

The lowest concentration of penicillin that completely inhibited growth as judged by the absence of turbidity after incubation for 18 hr at 37°C was taken to be the m.i.c.

Measurement of bactericidal penicillin concentrations

Bactericidal titres were estimated by adding an equal volume (2 ml) of a 1 in 50 dilution of a solution of penicillinase (prepared by Welcome Laboratories and containing 100,000 units of penicillinase per ml) to each of the tubes that showed no turbidity. A loopful of broth was then plated on to a well-dried blood agar plate and incubated for 18 hours at 37° C. The lowest concentration of penicillin that was sterile by this test was taken to be the bactericidal concentration.

Definition of a 'sensitive' strain of E. coli

The term 'sensitive' is obviously a relative one and will vary according to the organism and antibiotic employed and to the conditions under which they meet. When dealing with a large number of strains of bacteria and a single antibiotic any arbitrary level can be chosen, and organisms inhibited by this concentration of antibiotic may be termed 'sensitive' and the rest 'insensitive'. In a particular situation, for example the bladder, the level that would usually be chosen would be that which could be readily achieved by the antibiotic.

For reasons which are discussed on page 119 a penicillin concentration of 50 µg per ml has, for the purposes of this investigation been chosen to distinguish between strains that are 'sensitive' (i.e. they are inhibited by

this concentration of antibiotic) and those that are 'resistant' to penicillin.

Filter-paper disks impregnated with penicillin G

The greatest amount of penicillin in any of the available commercially-prepared disks was 5 units, equivalent to 3 μg. As this disk was clearly unsuited to distinguish between 'sensitive' and 'insensitive' categories of E. coli as defined above, disks containing varying amounts of penicillin per disk were produced and tested.

Production and testing of penicillin disks

A Pasteur pipette was used to deliver 0.02 ml per drop (Cruickshank, 1969 page 794) of appropriate dilutions of a freshly prepared solution of penicillin G on to sterile filter-paper disks, which were then dried in a vacuum. Disks containing 10, 25, 50, 100 and 200 μg of penicillin were thus prepared.

The disks were tested by placing them on well dried plates of nutrient agar, 3 mm deep, which had previously been spread with a culture of E. coli. In order to achieve a standard inoculum the strains of E. coli were grown overnight on a nutrient agar plate. Each culture was scraped off and resuspended in distilled water and calibrated in a nephelometer to give approximately 10^8 bacilli per ml. Two ml of a 1 in 100 dilution of this culture were flooded on to the nutrient agar plate and the excess removed immediately. Zone diameters were measured after incubation at 37°C for 18 hr.

At the same time, a tube-dilution sensitivity test was carried out on the same culture.

The disks were stored in a stoppered container without any dessicant and were used within one week. Some disks that had been stored for 10 weeks were re-tested and compared with newly prepared disks to determine the degree

of deterioration.

Following these experiments the 200 and 10 µg disks were discarded, and Oxoid Ltd, were approached and agreed to manufacture penicillin disks containing 25, 50 and 100 µg per disk. These disks were tested in the same way and the zone diameters compared with those obtained with a disk containing 25 µg of ampicillin (also supplied by Oxoid).

Use of penicillin disks

The penicillin sensitivity of each strain of bacteria from all the sources listed in Table 3 except the hospital strains, was determined using a filter paper disk containing 100 µg of penicillin. The technique used for these tests was to spread a single colony over a nutrient agar plate, apply the disk and incubate for 18 hours at 37°C. A strain was deemed to be 'sensitive' to penicillin when the diameter of the zone around the penicillin disk was 14 mm or more.

Sensitivity of the isolates to other antibiotics

All the bacteria, except some strains of Proteus and Ps. aeruginosa were tested against the following antibiotics in addition to penicillin, nalidixic acid, ampicillin, nitrofurantoin, kanamycin, colomycin, streptomycin, tetracycline, and sulphonamide. An Oxoid 'multodisk' was used for this purpose, each antibiotic being impregnated in a separate tip of the disk. The concentration of antibiotic in each disk is shown in Table 5, and in each case is that which is recommended by the manufacturer of the antibiotic for use when testing bacteria isolated from urinary tract infections.

TABLE 5

Antibiotics used in the Oxoid 'Multodisk'

Antibiotic	Quantity per disk (µg)	Antibiotic	Quantity per disk (µg)
Nalidixic acid	30	Streptomycin	25
Ampicillin	25	Colomycin	200
Nitrofurantoin	200	Tetracycline	50
Kanamycin	30	Sulphafurazole	500

Computer

In this work the computer was used to analyse the sensitivity patterns of all the organisms studied, and to correlate these with each other, with the biochemical type of the organism, and with the clinical circumstances of the patient from whom the organism was isolated. The clinical results were also processed by a computer.

The advantage of the computer in the research field is that it facilitates an investigation of many relationships which would not be possible by any other means.

Programme

The programme was written for an ICL computer which was in the process of being installed in the University of Dundee. The language most suited for medical and commercial data processing is COBOL. It was written on cards rather than magnetic or paper tape because, firstly this is the most suitable when it is desired to run the programme frequently with minor alterations, and secondly because cards are the simplest to use and are the most appropriate for an inexperienced programmer.

The programme is reproduced in full in Appendix one. It is a large and complex one involving the analysis of totally different types of data in its two basic parts - bacteriological and clinical. It could easily be modified to fit many different requirements.

Since the programme was modified slightly for almost every run, the programme reproduced in the appendix is a typical example. The modifications were small, for example there was no method by which the various age groups could be analysed and so this was done by running the programme once for each group, and on each occasion inserting a card which selected only the desired age group for examination. None of these 'selection cards' have been included in the appendix.

Prevalence of linked resistance

The computer was programmed to select the resistant isolates of each species and to calculate the percentage of them that were also resistant to each of the other antibiotics tested. If the association between (say) tetracycline and sulphonamide resistance was one of chance the percentage of sulphonamide-resistant strains that were also resistant to tetracycline would be the same as the percentage of sulphonamide-sensitive strains that were resistant to tetracycline. Where a significant difference between these two figures exists, some association or dissociation may be predicted between the resistances to the two antibiotics.

For example if 50 per cent. of sulphonamide-resistant strains were also resistant to tetracycline, but only 5 per cent. of sulphonamide-sensitive strains were tetracycline-resistant, then some association between resistance to each of the drugs might be presumed.

This association may be quantified in the form of a ratio; which is

$$\frac{\text{The percentage of sulphonamide-resistant strains that are resistant to tetracycline}}{\text{The percentage of sulphonamide-sensitive strains that are resistant to tetracycline}}$$

This ratio would be 10:1 in the example cited above. However it must be emphasized that this ratio will be meaningful only when the proportion of all strains resistant to either antibiotic is neither very small nor very large. Where there is complete cross-resistance the ratio will be infinity, and where the association is one of chance it will be unity, and where there is a dissociation between the two resistances it will be less than one.

Measurement of free penicillinase production

Strains of E. coli and Klebsiella were tested for penicillinase production by the method of Cruickshank (1969, page 904). A single colony of each strain was inoculated into 15 ml of nutrient broth and incubated for 18 hr at 37°C. The culture was sterilised by filtration through a 'Millipore' filter, the pore size being 0.45 μm. The filtrate containing any free penicillinase produced by bacteria under investigation was dispensed in tubes as indicated in Table 6. A solution of penicillin in nutrient broth was prepared. The concentration of penicillin varied according to the organism under investigation and was either 10, 100, or 1000 μg per ml. The penicillin solution was then added to the tubes already containing penicillinase as indicated in Table 6. All the tubes were not used in every estimation.

The test organism was the Oxford strain of Staphylococcus aureus. A single drop of a 1 in 100 diluation of this organism was added to each tube except the first immediately after the penicillin and penicillinase solutions were mixed together, and the rack of tubes was incubated without delay.

TABLE 6

Measurement of free penicillinase production

Number of tube	Amount of penicillin solution (ml)	Amount of penicillinase solution (ml)	Oxford Staph. aureus added *	Notes
1	0	2	-	Sterility control
2	0.25	2	+	
3	0.25	1	+	
4	0.5	1	+	
5	1	1	+	
6	2	1	+	
7	2	0.5	+	
8	2	0.25	+	Penicillinase diluted 1 in 10 with nutrient broth
9	2	1	+	
10	2	0.5	+	
11	2	0.25	+	
12	2	0.1	+	
13	2	0.05	+	
14	0	2	+	Control showing growth of test organism

* + indicates addition of the test organism, - indicates its omission.

The quantity of penicillin destroyed per ml of penicillinase is calculated from the tube with the least proportion of penicillinase showing growth after 18 hours incubation at 37°C. Thus if the penicillin solution contained 100 µg of penicillin per ml and growth (indicated by turbidity) occurred in tubes 2 - 7 and 14, the quantity of penicillinase per ml would

be the equivalent of 400 µg of penicillin.

With each batch of tests the penicillin sensitivity of the control strain of Staph. aureus was confirmed by tube-dilution.

One-step mutants

This experiment was a repeat of the experiment carried out by Barber in 1949 with strains of Staphylococcus aureus in which she demonstrated that penicillin-resistant strains that were stored on the bench gave off penicillin-sensitive mutants. In the experiments reported below a number of resistant strains of E. coli were stored and tested at intervals to see whether 'one-step' mutations to the penicillin sensitive state occurred. Sensitive strains were also stored and tested for resistant mutations.

Seven penicillin-resistant and eight penicillin-sensitive strains were selected from the hospital strains of E. coli. The penicillin sensitivity of, and penicillinase production (where measured) by each strain is shown in Table 7.

TABLE 7

Penicillin sensitivity of, and penicillinase production by strains used in the 'one-step' mutation experiment

Number of strain(s)	Penicillin sensitivity (µg per ml)	Penicillinase production (µg of penicillin destroyed per ml of penicillinase)
17, 34, 48, 70	25	NT
31, 40	25	<0.1
56	50	<2.5
81	50	NT
44	500	100
35	1000	10
11, 21, 63, 79	1000	NT
4	2500	NT

NT - Not tested.

A single colony of each strain was inoculated into two bottles of nutrient broth which were incubated for 18 hours at 37°C. One culture was then stored at 4°C in a refrigerator, and the other was stored on a shelf at room temperature which fluctuated widely.

At intervals during the subsequent year each culture was streaked on to nutrient agar plates in order to obtain single colonies, and the penicillin sensitivity of 50 colonies was tested on ditch plates.

Preparation of the ditch plates. Six-inch glass Petri dishes were poured with nutrient agar. One 100 ml bottle of molten agar was cooled to 45°C and 5 ml of nutrient broth containing 10,000 µg of penicillin per ml was added, so that the concentration of agar was approximately 500 µg per ml. This agar was poured into a ditch cut in the agar plate. The plates were then stored in the refrigerator over night to allow pre-diffusion of the penicillin. About 25 strains could be tested on each plate (see Figure 3) Strains which appeared to show a change of sensitivity were tested by tube dilution.

The ditch plate was tested by using 16 colonies of a penicillinase-producing strain of E. coli, and one colony of a sensitive non-penicillinase-producing strain to confirm that the streaks were not so close that the penicillinase from the resistant colonies influenced the apparent sensitivity of the sensitive strain. There was no such interference.

Training bacteria to penicillin resistance

Six strains of E. coli were selected from the strains isolated from patients in hospital, and the sensitivity of each to penicillin confirmed by tube-dilution. Strain number 35 was inhibited by 2500 µg of penicillin per ml, numbers 4 and 44 by 1000 µg per ml, number 31 by 25 µg per ml, and rough

FIGURE 3A

Ditch Plate

Six inch diameter Petrie dish with a ditch containing penicillin. Twenty five colonies from a single strain have been tested, all are penicillin sensitive. Magnification x 0.66.

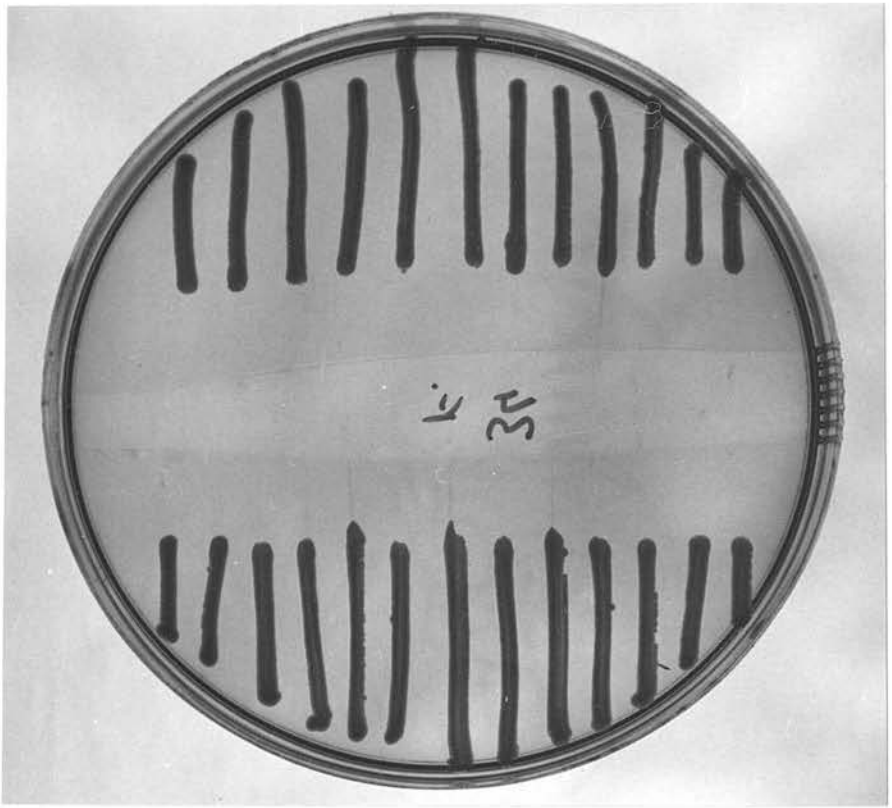
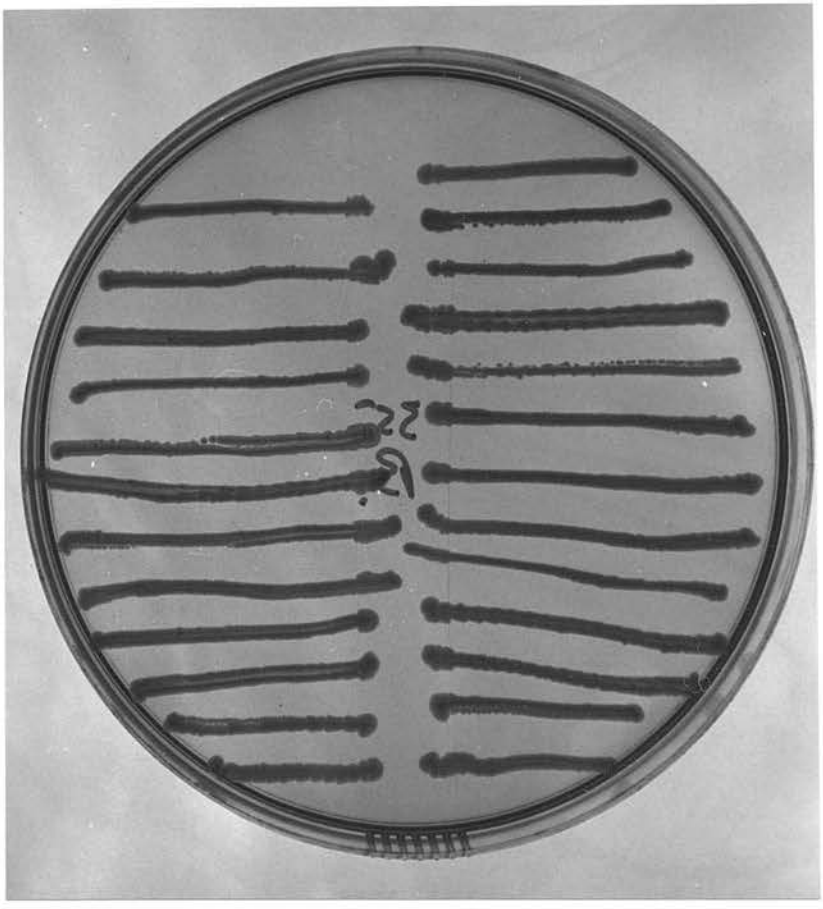


FIGURE 3B

Ditch Plate

Six inch diameter Petrie dish with a ditch containing penicillin. Twenty five colonies from a single strain have been tested, all are penicillin resistant. Magnification x 0.66.



and smooth variants of strain number 40 (40R and 40S) were both inhibited by 50 µg of penicillin per ml.

Tubes of nutrient broth containing 10, 25, 50, 100, 250, 500, 1000, 2500, 5000, 10,000 and 25,000 µg of penicillin per ml were prepared.

At the beginning of the experiment a single drop of a 1 in 100 dilution of an overnight broth culture in 0.85 N NaCl was added to an appropriate range of tubes, comprising some with a lesser and some with a greater concentration of penicillin than the expected inhibitory level, and a control tube of nutrient broth only. After incubation for 18 hours at 37°C the mean inhibitory level was recorded, and a 1 in 100 dilution in 0.85 N NaCl of the culture containing the greatest concentration which showed growth was used to inoculate a further series of tubes. At each step a loopful of the culture was plated on to MacConkey agar and nutrient agar to check the colonial morphology and purity of the strain.

After 17 sub-cultures in increasing concentrations of penicillin the ability of the original and trained strains to produce penicillinase was tested (see page 92), and these strains were stored on Dorset's egg slopes. The trained strains were then serially sub-cultured 20 times on penicillin-free medium, and following this the penicillin sensitivity, and ability to produce penicillinase were tested again. These strains were stored on Dorset's egg slopes. After nearly two years the penicillin sensitivities of the stored strains were determined once more.

The model urinary bladder

The model bladder consisted of a 500 ml flask in an incubator at 37°C. At the beginning of the experiment a quantity of 'residual infected urine' was added to the flask. This was prepared by sterilising an aliquot

of urine (by Seitz filtration) inoculating it with a strain of E. coli and incubating it overnight.

It was desirable that the model bladder should mimic, as closely as possible the conditions of urine volume, urine concentration of penicillin, and total amount of penicillin excreted, which had been shown to exist in vivo (see page 102). However for convenience and for comparison with experiments carried out by other authors (O'Grady and Pennington, 1967; Greenwood and O'Grady, 1969) a rate of urine excretion into the model of one ml per minute, sixty ml per hour was required. Since the mean excretion of urine, over 6 hours of the six subjects taking penicillin G was only 46 ml per hour (see Table 9) it was not possible to mimic both the concentration of penicillin in the urine, and the total amount of penicillin excreted, Two courses of action were possible - either to equate the urine concentrations, which would result in the use of more penicillin than the mean that was excreted by the six subjects, or to equate the total penicillin excretion over six hours resulting in a reduction in the concentration of penicillin per ml. The first course was chosen, and the total amount of penicillin used in the model bladder every six hours was 92,025 µg, compared with the mean excretion by the six subjects which was 67,281 µg (see Table 13).

An aliquot of 15 ml of warmed sterile urine was added to the model bladder every 15 minutes. The quantity of penicillin in each aliquot was calculated in the light of the knowledge of excretion of penicillin in urine (MacDermott, et al., 1946), so that over each two hour period the concentration of penicillin in the relevant eight aliquots equalled that recorded in vivo. The amount of penicillin, and its concentration in each aliquot is recorded in Table 8, and illustrated in Figure 4, and the mean concentration of

penicillin in the two hourly specimens from the six subjects is recorded in Table 12.

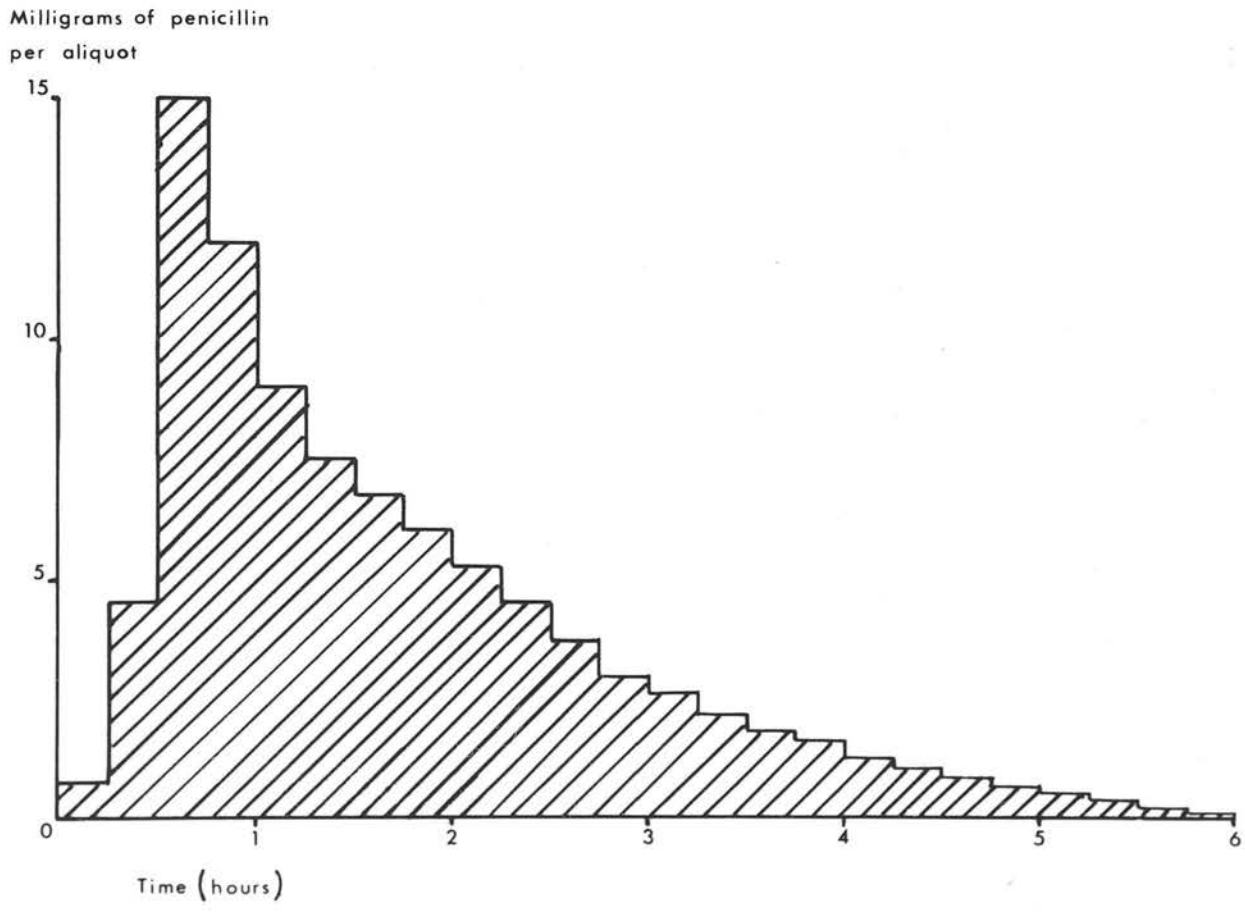
TABLE 8

The amount of penicillin G and its concentration in each of 24 15 ml aliquots of warmed sterile urine added to a model bladder at intervals after the experiment was begun

Time after experiment started	Amount of penicillin (mg per 15 ml aliquot)	Concentration of penicillin in each aliquot (µg per ml)
0	0	0
¼	750	50
½	4500	300
¾	15,000	1000
1	12,000	800
1¼	9000	600
1½	7500	500
1¾	6750	450
2	6000	400
2¼	5250	350
2½	4500	300
2¾	3750	250
3	3000	200
3¼	2625	175
3½	2250	150
3¾	1875	125
4	1650	110
4¼	1350	90
4½	1125	75
4¾	900	60
5	750	50
5¼	600	40
5½	450	30
5¾	300	20
6	150	10

FIGURE 4

Amount of penicillin in each of twenty four aliquots of urine added to the model bladder


Milligrams of penicillin
per aliquot
15
10
5
0
1
2
3
4
5
6
Time (hours)

Viable counts

The viable count of the organisms present in the 'residual infected urine' was estimated by the technique of Miles and Misra, (Miles, Misra and Irwin, 1938). Ten fold dilutions of the urine were made in 0.85 N NaCl with a fresh pipette for each dilution. A sterile pipette that had been calibrated so that it delivered 1/32nd ml per drop (Cruickshank, 1969, page 794) was used to place four drops of each dilution on to a well dried blood agar plate. A single pipette was used for each viable count, beginning with the greatest dilution. The smallest number of organisms which could be detected by this method was 8 bacteria per ml (one colony from the four drops). A 'sterile' urine therefore was one with less than eight bacteria per ml.

At intervals during the experiment, and again at the end, the flask was shaken, 1 - 2 ml were withdrawn and the number of organisms counted in the same way. When the experiment lasted for longer than 6 hours the flask was 'voided' at the end of the sixth hour leaving behind the same amount of residual urine as at the beginning of the experiment.

Bacteria

The experiments were performed initially with a strain of *E. coli* sensitive to 25 µg of penicillin G per ml (measured by tube-dilution), and later with a strain of *E. coli* sensitive only to 1000 µg of penicillin per ml. In experiments with the sensitive strain residual volumes of 10, 50 and 100 ml were added to the flask at the beginning of the experiments, but in those with the resistant strain residual volumes of 1 and 10 ml only were employed. The amount of penicillinase produced by the resistant strain of *E. coli* was measured by the method modified from that of Cruickshank (1969) as described on page 92.

As a control, experiments in which 15 ml aliquots of urine without penicillin were added to residual volumes of 1 and 10 ml were performed to test the effect of simple dilution and voiding six hourly.

Control experiment

A simple control experiment was performed in which the effect of the addition of an equal volume of sterile (filtered) urine containing penicillin to an overnight culture in urine of a strain of E. coli (sensitive to 25 µg of penicillin per ml) was assessed. The experiment was performed four times, and the amount of penicillin in the aliquots of sterile urine was adjusted so that the final concentrations in the four experiments were 50, 100, 250 and 500 µg per ml.

The cultures were incubated at 37°C, and sampled at intervals of ½, 2, 4 and 6 hours.

RESULTS

URINARY EXCRETION OF PENICILLIN G

Levels of penicillin in blood and urine

The levels of penicillin in the blood and urine after oral administration of the three preparations of penicillin G are recorded in Table 9. Penicillin concentrations of, or in excess of, 512 µg per ml were regularly observed in the specimen of urine taken after 2 hours, and with potassium penicillin G the urine concentration at this time was remarkably constant at that level. Differences of up to 8-fold in the concentration of penicillin in the urine between subjects examined at the same time were observed.

Serum concentrations of penicillin were approximately 1000 times less than the urine levels, and they were less variable than the urine levels, especially following penamicillin. On three occasions the serum level was undetectable (less than 0.02 µg per ml) after 6 hours. Two of these occasions followed administration of sustained action penicillin G and and were associated with low levels of penicillin in the serum (0.16 µg per ml) two hours after the dose, and the third followed potassium penicillin G and was associated with an unusually high level in the second hour (1.28 µg per ml).

The patients can be conveniently allocated into three groups according to their urine output in the first six hours. Subjects JH and JE had an output of more than 400 ml on each occasion, BH and AB had outputs of 215 ml or less on each occasion, and SH and DG were intermediate in position, although DG had a high urine output (460 ml) when taking penamicillin.

TABLE 9

Levels of penicillin in urine and blood at different times after oral administration of a single dose of one of three preparations of penicillin G

Drug and dose	Subject	Level of penicillin in urine (μg per ml) collected at time (hr) after drug given by mouth						Volume of urine passed between 0 and 6 hr (ml)	Level of penicillin in blood serum (μg per ml) at time (hr) after drug given by mouth	
		2	4	6	8	12	24		2	6
Potassium penicillin G 500 mg	JH	512	512	64	16	0	0	400	1.28	<0.02
	BH	512	256	32	16	8	0	190	0.16	0.02
	AB	512	128	64	32	8	0	215	0.64	0.04
	DG	1024	256	32	8	2	0	255	0.32	0.02
	JE	512	64	32	8	2	0	410	0.64	0.04
	SH	512	128	16	4	0	0	180	0.32	0.04
Penamecillin 700 mg equivalent to penicillin G 610 mg	JH	128	512	64	16	2	0	560	0.64	0.08
	BH	1024	256	32	16	8	2	165	0.64	0.04
	AB	1024	512	64	32	8	4	165	0.64	0.04
	DG	256	128	16	16	8	0	460	0.64	0.04
	JE	256	128	64	32	8	4	440	0.64	0.08
	SH	512	512	128	32	8	2	335	0.64	0.04
Penicillin G sustained action 450 mg	JH	256	128	32	8	2	0	495	1.28	0.08
	BH	512	128	64	8	2	0	200	(contaminated)	
	AB	512	256	64	32	0	0	190	0.16	<0.02
	DG	64	128	32	8	2	0	385	0.32	0.04
	JE	64	32	8	2	0	0	580	0.16	<0.02
	SH	512	128	64	8	0	0	230	0.16	0.02

TABLE 10

Measured excretion of penicillin G at different times after taking an oral dose

Subject	Quantity of penicillin (μg) excreted in the urine when the drug and dose was								
	penicillin G 500 mg after (hr)			penamicillin 700 mg equivalent to penicillin G 610 mg after (hr)			sustained action penicillin 450 mg		
	2	6	24	2	6	24	2	6	24
JH	51,200	110,600	112,500	23,000	86,000	91,200	35,800	57,300	60,000
BH	33,250	54,050	56,580	67,500	84,180	86,580	35,800	51,800	52,500
AB	15,400	32,100	36,300	20,480	54,450	58,550	30,700	47,840	50,435
DG	72,000	100,300	102,000	33,300	50,920	55,320	14,100	26,600	27,750
JE	35,800	55,320	56,470	38,400	68,150	74,750	19,200	29,230	29,950
SH	30,800	39,440	39,840	69,000	156,120	161,200	61,600	72,480	73,040

There does not seem to be any relation between urine output and the measured level of penicillin in the urine.

Table 10 shows the measured excretion of penicillin after 2, 6 and 24 hours for each of the patients. The most striking feature is the variability not only between different subjects taking the same drug, but also when different drugs were taken by the same subject. For example there is almost a threefold difference between the least and the most of each drug excreted, and a fourfold difference (without accounting for the slightly different dose) between SH's urinary excretion of potassium penicillin and penamicillin.

There is little evidence to show that a subject who excretes one drug well will excrete all the others well also. Subject JH had a consistently high level of excretion, and subject AB a consistently low level, but otherwise there seemed to be no pattern.

Conclusions drawn from one subject invalid

Two factors make it difficult to draw any firm conclusions by comparing the results of any one subject with any one other, or with the mean. The stepwise nature of the measurements, so that a difference of a few µg in the amount of penicillin in the urine, or perhaps a difference of a few minutes in the precise time of the collection of the specimen of urine may double or halve the result of a single measurement. Secondly since approximately half the total excretion of penicillin occurs in the first two hours (see Table 11) a difference of only one tube in this result will make a profound change in the total.

TABLE 11

Rapidity of penicillin excretion in the urine

Preparation of penicillin	Proportion (%) of the mean total excretion of penicillin over 24 hours which was excreted by (hours)		
	2	4	6
Potassium penicillin	59	89	97
Penamicillin	48	87	91
Sustained action penicillin	68	91	98

Rapidity of excretion of penicillin

Table 11 illustrates the proportion of the total amount of penicillin excreted which was completed in the first six hours after administration of an oral dose. The sustained action penicillin was excreted most rapidly, and penamicillin least rapidly, but for all three drugs, nearly nine tenths of the excretion had taken place in the first four hours, and by the sixth hour it was substantially completed.

Mean concentrations of penicillin in urine and blood

The arithmetic mean of the estimations of the concentration of penicillin in urine and blood (listed in Table 9) was calculated for each antibiotic at each period after the administration of the dose. These figures were adjusted, for penamicillin and sustained action penicillin, to a dose of 500 mg and they are recorded in Table 12.

If a concentration of 50 µg of penicillin per ml of urine be considered the minimum required to inhibit sensitive strains of E. coli (see page 119) then reference to Tables 9 and 12 shows that, after an oral dose of 500 mg of penicillin G, this concentration will certainly be achieved for the first four hours, and is very likely to be attained during much of the fifth and sixth hours in the ureteric urine. If bladder voiding is timed to co-incide with each administration of the dose, a concentration very much in excess of 50 µg of penicillin per ml, could be maintained indefinitely in the bladder. That there is considerable variation between different subjects in the concentrations of penicillin achieved cannot be disputed but for most of the first six hours this is unlikely to be important therapeutically even in the upper urinary tract.

TABLE 12

Mean concentrations of penicillin calculated from the results in Table 9 and adjusted to a 500 mg dose of penicillin given by mouth

Drug	Adjusted mean concentration of penicillin in urine (µg per ml) at time (hr) after drug given						Adjusted mean concentration of penicillin in serum (µg per ml) at time (hr) after drug given.	
	2	4	6	8	12	24	2	6
Potassium penicillin G	597	324	40	14	4	0	0.56	0.03
Penamicillin	437	280	52	21	8	0	0.52	0.04
Sustained action penicillin G	350	150	49	13	1	0	0.47	0.03

Amount of penicillin excreted in urine

The amount of penicillin excreted in the urine during the first twenty four hours after the administration of the three preparations of penicillin to the six subjects are set out in Table 13. The mean urinary excretion of potassium penicillin G was 13.4 per cent. of the dose, for penamicillin it was 14.1 per cent., and for sustained action penicillin it was 11.1 per cent.

TABLE 13

Urinary excretion of penicillin over 24 hr in six subjects after taking a single dose of one of three different preparations of penicillin G by mouth

Value	Value for experiment in which drug (and dose, mg) was		
	Potassium penicillin G (500)	Penamicillin (700) equivalent to penicillin G (610)	sustained action penicillin G (450)
Mean measured excretion (µg)	67,281	86,185	49,922
Percentage of dose excreted	13.4	14.1	11.1
Mean measured excretion (µg) adjusted to a dose of 500 mg	67,281	70,402	54,360

Miscellaneous estimations of penicillin concentrations in urine

The results of some further estimations of penicillin concentrations in urine are recorded here because their interest now lies in the differences which the altered experimental circumstances may have produced in the pattern of excretion, and in the comparison of the results from patients with urinary infection with those from the healthy subjects whose excretion of penicillin has been analysed above.

TABLE 14

Penicillin concentration in the urine of a single subject after oral administration of 500 mg post-prandially

Time (hr) between breakfast and the administration of penicillin	Concentration of penicillin in urine (μg per ml) at time (hr) after drug given by mouth					
	1	2	3	4	5	6
1	20	20	160	640	320	640
1	<20	20	640	320	160	40
3	80	160	160	320	160	160

Table 14 records the results of the experiments in which oral penicillin was taken post-prandially. The alteration in the pattern is clear, with low concentrations of penicillin in the first 2 hours, and higher levels subsequently. The results of this very limited experiment do not suggest that taking penicillin post-prandially affects the actual concentrations achieved in the urine, but the timing was altered.

When the dose of penicillin was only 250 mg (taken pre-prandially) the concentrations of penicillin in the urine (Table 15) were, as expected, lower and did not rise high enough to ensure that the concentration of 50 μg per ml would be exceeded for most of the first six hour period after administration of the drug.

TABLE 15

Concentration of penicillin in the urine after pre-prandial oral administration of 250 mg of potassium penicillin G

Time (hr) after drug given by mouth	2	4	6	8	12	24
Concentration of penicillin (μg per ml)	32	128	32	8	2	0

In Table 16 the concentration of pencillin in the urine of an adult female patient following the first dose of oral potassium penicillin G for an acute urinary tract infection is recorded. In Table 17 the measured levels of penicillin in the urine of a $2\frac{1}{2}$ year old male child are set out.

TABLE 16

Concentration of penicillin in the urine of a patient suffering from a urinary tract infection following oral administration of 500 mg of potassium penicillin G

Time (hr and min) after drug given by mouth*	0.30	1.10	2.00	3.00	5.05	15.00
Concentration of penicillin (μg per ml)	...	256	128	32	64	8

* The bladder was emptied at the time that the drug was given.

The dose (125 mg) given to the child was proportionately much larger than that given to adults, but the general pattern of penicillin concentrations, high initially and falling off later was similar to that noted in adults. The penicillin was given immediately after the child had passed urine in the morning, and before breakfast.

TABLE 17

Concentration of penicillin in the urine of a child at different times after pre-prandial oral administration of 125 mg of potassium penicillin G (half of one crushed tablet of 'Crystapen G')

Day of treatment	Concentration of penicillin in urine (µg per ml) at time (hrs and min) after drug given by mouth					
	1hr 45 min	2hr 45min	3hr 20min	5hr 00min	5hr 10min	6hr 00min
2	640*	...	...	...	320	...
3	...	...	640	...	...	160
4	...	320	...	160	...	...

* On day 2 this was the greatest amount of penicillin that could be detected by the test.

PENICILLIN FILTER-PAPER DISK

Initial testing of filter-paper disks prepared in the laboratory

Tables 18 and 19 show the results of testing the penicillin disks with 24 strains of *E. coli*. Two tables are necessary: (1) because two batches of disks were used, and there appeared to be some differences between them; and (2) because two of the five disks used in the first batch were omitted from the second.

TABLE 18

Five penicillin disks tested with 13 strains of *E. coli*

Sensitivity of strains of *E. coli* (µg per ml)	Number of strains	Mean zone diameter (and range) in mm for each of the five penicillin disks (µg per disk)				
		10	25	50	100	200
25	1	0	0	16	18	25
50	5	0	0	12(0-17)	13(0-18)	22(17-25)
100	5*	0	0	5(0-15)	10(0-17)	19(17-25)
>300	2	0	0	0	0	0

* One strain was inhibited by 150 µg per ml, but not 100 µg per ml

Table 18 records the results with the first batch of disks. Clearly a disk containing only 10 µg per ml is unsuitable, and probably a disk with 25 µg per ml is also unsuitable. Furthermore the 200 µg disk failed to distinguish between those strains that were sensitive to 50 µg per ml, and

those that required 100 or 150 µg per ml for inhibition.

At this stage therefore the 10 µg disk was discarded as unsuitable. In the interests of caution, and because those strains that were inhibited only by 100 µg of penicillin per ml might tend to become rapidly more resistant to penicillin (although this has not been demonstrated) it was considered advisable to arrange the disk strength to exclude these strains, and the 200 µg disk was discarded as well.

TABLE 19

Three penicillin disks tested with 11 strains of *E. coli*

Sensitivity of strains of *E. coli* (µg per ml)	Number of strains	Mean zone diameter (and range) in mm for each of the three penicillin disks (µg per disk)		
		25	50	100
25	7	12 (11-17)	18 (15-21)	22 (20-25)
50	2	13 (12-14)	19 (18-20)	23 (22-24)
>300	2	0	0	0

It is clear when Tables 18 and 19 are compared that there was a considerable difference between the two batches of disks. This is demonstrated in Table 20 in which the disks used in Table 18, which by the time of this test were 10 weeks old, were compared with the disks used in Table 19 (which were less than 2 weeks old).

Although the results from Tables 18, 19 and 20 are difficult to compare because of the small number of strains in each group, two tentative conclusions were made: (1) There was not much deterioration over 10 weeks despite the unsatisfactory method of storage (without a dessicant); and (2) The two batches differed significantly in strength, and that this inconsistency was sufficiently great to render the laboratory prepared disks unsuitable for clinical use.

TABLE 20

Comparison of new and old penicillin disks

Sensitivity of strains of *E. coli* (µg per ml)	Number of strains	Mean zone diameter (and range) in mm for each of the disks which contained 50 µg of penicillin per disk	
		New*	Old
25	6	19 (17-21)	15 (12-19)
50	2	19 (18-20)	15 (12-17)
>300	2	0	0

* The same results as those used for Table 19.

Commercial penicillin filter-paper disks

Oxoid Ltd were therefore approached and agreed to manufacture disks of three strengths; 25, 50 and 100 µg per disk. These disks were tested in the same way, and a filter-paper disk containing 25 µg of ampicillin was included in the test for comparitive purposes. This disk is recommended

by Oxoid Ltd for testing bacteria causing urinary tract infection.

The distinction of sensitive and resistant strains with filter-paper disks impregnated with antibiotic is obviously most satisfactory when sensitive strains have a wide zone and resistant strains no zone at all. Even in borderline cases judgement is often made quickly and usually without accurate measurement (which may not be justified in individual cases anyway). A zone diameter of twice the diameter of the disk is normally considered to be satisfactory, and so in the experiments reported above where the disk diameter was 7 mm, the criterion for judging a strain to be sensitive would be a diameter of 14 mm.

TABLE 21

Comparison of penicillin and ampicillin disks prepared by Oxoid Ltd

Sensitivity of strains of *E. coli* (µg per ml)	Number of strains	Mean zone diameter (and range) in mm for each disk			
		Penicillin (µg per disk)			Ampicillin (25 µg per disk)
		25	50	100	
25	25	11 (8-15)	16 (12-20)	20(15-24)	26 (20-30)
50	14	8 (0-13)	14 (10-17)	17(13-22)	23 (14-28)
100	3	0	8 (0 -12)	13*(10-15)	17 (12-20)
>300	11	0	0	0	0**

* Only two strains tested

** One strain had a zone diameter of 12 mm, all the others had no zone.

In Table 21 the results of tests with the disks prepared by Oxoid Ltd are recorded. The 25 µg disk is clearly unsuitable. With the 50 µg disk 10 of the 39 strains sensitive to 25 or 50 µg of penicillin per ml by tube dilution tests had zones of 13 mm or less. Only two of these strains had a zone of 13 mm (none had a smaller zone) with the 100 µg disk. This disk was therefore selected and manufactured by Oxoid Ltd in some quantity.

PENICILLIN SENSITIVITY OF GRAM-NEGATIVE BACILLI

Eighty three per cent. of the 969 strains of bacteria in the investigation were sensitive either to 50 µg of penicillin per ml by the tube-dilution test, or to the special filter-paper disk impregnated with 100 µg of penicillin. The proportion of sensitive strains in each of the various groups of bacteria is shown in Table 22. Eighty six per cent. of all the strains of *E. coli* and 94

TABLE 22

Penicillin sensitivity of the strains of bacteria

Source	Percentage (and number) of strains sensitive to penicillin in each species or group							
	All species	*E. coli*	*Klebsiella*	Other coliforms	*Proteus*	Other Gram-negative bacilli	Gram-positive cocci	
							Staph. aureus	*Str. faecalis*
Hospital	77 (243)	76 (108)	23 (17)	33 (6)	98 (100)	0 (12*)	... ...	... ...
General practitioner	80 (502)	81 (305)	84 (26)	65 (20)	86 (57)	63 (60)	100 (4)	86 (30)
Antenatal clinic	100 (48)	100 (38)	... (0)	... (0)	100 (3)	100 (6)	100 (1)	... (0)
Faeces from healthy adults	96 (176)	96 (157)	100 (14)	60 (5)	... ...	... ...	... ...	... ...
Total	83 (969)	86 (608)	70 (57)	58 (31)	94 (160)	56 (78)	100 (5)	86 (30)

* All were strains of *Ps. aeruginosa*.

per cent. of all Proteus strains were sensitive, as were all the strains of Staphylococcus aureus and 86 per cent. of strains of Streplococcus faecalis. Other groups of bacteria were less sensitive, the unidentified coliform organisms, and the non-lactose fermenting Gram-negative bacilli other than Proteus having only 58 per cent. and 56 per cent. of strains, respectively, sensitive to penicillin G.

Every one of the 48 consecutive isolates from patients with bacteriuria of pregnancy, and 80 per cent. of the 502 consecutive isolates from general practice patients were sensitive to penicillin.

Detailed bactericidal and bacteristatic penicillin sensitivity tests

The results of the tube-dilution sensitivity tests and of the tests to determine the mean bactericidal concentration of penicillin of the hospital strains of Gram-negative bacilli are illustrated in Table 23. All strains that were included in the bactericidal test were also included in the bacteristatic test. Seventy six per cent. of strains of E. coli, and the 98 per cent. of strains of Proteus including all the strains of Proteus mirabilis were inhibited by 50 µg of penicillin per ml. All strains of E. coli which were inhibited by 50 µg per ml were killed by 150 µg per ml.

The different proportions of strains of E. coli and of strains of Klebsiella and other coliform bacilli which were inhibited by 50 µg per ml is striking. Nearly 80 per cent. of strains of E. coli were sensitive to 50 µg per ml, whereas 80 per cent. of strains of Klebsiella and 71 per cent. of strains of other coliform bacilli were resistant to this figure. Strains of Proteus were much more sensitive to penicillin than were strains of E. coli, only two strains requiring more than 50 µg for inhibition.

TABLE 23

Detailed bactericidal and bacteristatic penicillin sensitivities of strains of Gram-negative bacilli isolated from patients in hospital

Species or group of bacteria	Test	Number of strains	Percentage of strains inhibited or killed by different concentrations of penicillin G (μg per ml)			Percentage of strains unaffected by 150 μg of penicillin G per ml
			5	50	150	
E. coli	m.i.c.	108	0	76	82	18
	m.b.c.	81	0	59	80	20
Klebsiella	m.i.c.	10	0	20	20	80
	m.b.c.	7	0	0	14	86
Other coliform bacilli	m.i.c.	14	0	29	36	64
	m.b.c.	13	0	23	31	69
Proteus**	m.i.c.	100	88	98*	...	...
	m.b.c.	100	46	91*	...	...

Notes: m.i.c. - Mean inhibitory concentration

m.b.c. - Mean bactericidal concentration

* The remaining strains (2 for the inhibitory test, and 9 for the bactericidal test) were unaffected by 50 μg per ml of penicillin and were not tested further.

** Ninety seven strains were Proteus mirabilis, and three were Proteus vulgaris. Two of the latter three strains were inhibited and killed by 5 μg of penicillin per ml, the other required 50 μg per ml.

Penicillin sensitivity of resistant strains of Gram-negative bacilli

Those strains of E. coli, Klebsiella and Ps. aeruginosa that were unaffected by 150 µg of penicillin per ml usually required 1000 µg or more for inhibition (Table 24). There appears to be therefore a clear division of these species of Gram-negative bacilli into a penicillin-sensitive group, most of which are inhibited by 50 µg per ml, and probably all by 150 µg per ml, and a penicillin-resistant group requiring ten times that concentration of penicillin for inhibition.

TABLE 24

Penicillin sensitivity of resistant strains of Gram-negative bacilli

Species of bacteria	Number of strains	Number of strains sensitive to different concentrations of penicillin (µg per ml)					
		250	500	1000	2500	5000	10,000
E. coli	18	0	4	13	0	1	0
Klebsiella	7	0	1	1	1	4	0
Other coliforms	7	0	0	5	2	0	0
Ps. aeruginosa	12	0	0	2	4*	6	0

* Four strains insensitive to 1000 µg per ml and not tested further.

Antibiogram of the bacteria investigated

The results of the antibiotic sensitivity tests carried out with the Oxoid

multodisk are shown in Table 25. The most notable fact is the very high proportion of strains that are sensitive to each antibiotic. For the species E. coli the drug that inhibited the least number of strains was sulphonamide, followed by penicillin, tetracycline and ampicillin, whereas nalidixic acid, nitrofurantoin, kanamycin and colomycin were active against virtually every strain.

The hospital strains of Klebsiella had the most highly developed resistance pattern with only 23 per cent. sensitive to penicillin and 29 per cent. to ampicillin, and a lower proportion of strains sensitive to each of the other antibiotics (except colomycin and sulphonamide) than strains of E. coli. The six other lactose-fermenting coliform bacilli showed a similar high proportion of strains resistant to penicillin, ampicillin, streptomycin, tetracycline and sulphonamide.

The strains of Proteus showed an expected resistance to colomycin and tetracycline, and the five strains of Staphylococcus aureus showed a similar resistance to colomycin.

The most resistant species was Streptococcus faecalis with only a quarter of the strains (approximately) sensitive to nalidixic acid and sulphonamide, and about half resistant to streptomycin, kanamycin and colomycin, but a high proportion were sensitive to ampicillin (96 per cent.) and penicillin (86 per cent.).

Exposure to antibiotics and penicillin sensitivity

As the likelihood of recent exposure to antibiotics increased, so the proportion of strains that were penicillin-sensitive decreased. This is illustrated in Table 26.

TABLE 25

The proportion of strains of each species or group of bacteria from each source which was sensitive to each of 9 antibiotics

Species or group of bacteria	Source*	Number of strains	The proportion (%) of strains sensitive to								
			nalidixic acid	ampicillin	nitrofur-antoin	kanamycin	colomycin	tetra-cycline	strepto-mycin	sulphon-amide	penicill-in
E. coli	Hosp.	100	99	83	97	100	94	79	81	68	76
	G.P.	305	98	84	98	96	94	81	82	70	81
	A-N	35	100	100	100	100	100	100	100	100	100
	Faeces	156	100	98	99	100	99	96	96	91	96
Klebsiella	Hosp.	17	82	29	88	88	94	64	52	70	23
	G.P.	26	100	78	100	94	89	84	94	88	84
	Faeces	14	100	100	100	100	100	100	100	100	100
Other coliforms	Hosp.	6	100	50	100	100	100	50	50	33	33
	G.P.	20	95	55	75	95	100	85	95	85	65
	Faeces	5	100	60	100	100	100	100	100	80	60
Proteus	G.P.	57	91	82	85	94	17	17	85	61	86
	A-N	3	66	100	33	100	0	0	100	0	100
Other non-lactose-fermenting organisms	G.P.	60	85	68	78	91	73	76	85	66	63
	A-N	6	100	100	100	100	83	83	83	83	100
Str. faecalis	G.P.	30	26	96	76	53	46	66	46	20	86
Staph. aureus	G.P. A-N	5	100	100	100	100	20	100	100	40	100

* Hosp. - Strains isolated from patients in hospital
G.P. - Strains isolated from patients in the community
A-N - Strains isolated from patients attending an antenatal clinic
Faeces - Strains isolated from the faeces of healthy adults.

TABLE 26

Proportion of strains that were penicillin sensitive and proportion of strains that were E. coli

Group	Proportion (%) of strains that were penicillin sensitive	Proportion (%) of lactose-fermenting strains that were *E. coli*	Proportion (%) of all strains that were *E. coli*
Hospital series, (lactose-fermenting strains)	77	81	...
General practice I, recent urinary infection	74	85	58
General practice II, no recent urinary infection	91	90	66
Antenatal series	100	100	79
Faecal strains from healthy adults, (lactose-fermenting strains)	96	89	...

Of the faecal strains from healthy adults, and the strains from the antenatal clinic 96 per cent. and 100 per cent. respectively were sensitive to penicillin. Of the hospital strains only 77 per cent. were sensitive. The strains from general practice were divided into two groups; those obtained from patients who had a history of recent or recurrent urinary tract infection; and those who had no such a history. No account was taken of antibiotics given for other conditions. Included in the first group were strains isolated from patients as a (failed) test for cure. Seventy four

per cent. of the first group were penicillin-sensitive, and 91 per cent. of the second group.

Exposure to antibiotics and the proportion of strains of *E. coli*

The proportion of urinary strains that were *E. coli* decreased with penicillin-sensitivity as exposure to antibiotics and the hospital environment increased, (Table 26). In the consecutive series studied this proportion fell from 79 per cent. of the antenatal specimens to 58 per cent. of specimens from patients with a history of previous urinary infection under the care of a general practitioner.

This finding was mirrored by a similar decrease in the proportion of lactose-fermenting strains that were *E. coli* from 100 per cent. of the antenatal specimens to 81 per cent. of the hospital specimens, and there was a consequent increase in the number of strains of *Klebsiella* and other unidentified lactose-fermenting strains.

Reduction in antibiotic sensitivity following exposure to antibiotic

The correlation between the antibiotic sensitivity of the bacteria and a history of urinary infection was not confined to penicillin. The sensitivities of the specimens received from the general practitioners, to the eight antibiotics on the Oxoid 'Multo-disk' (see Table 5) was related to a history of recent infection. Table 27 records the results for penicillin, sulphonamide, tetracycline, ampicillin and streptomycin. The proportion of strains in both groups sensitive to kanamycin, colomycin, nitrofurantoin and nalidixic acid was similar.

The reduction in the proportion of strains sensitive to various antibiotics

TABLE 27

Antibiogram of bacterial isolates and exposure of the patient to antibiotics for urinary infection

Antibiotic tested	Recent urinary infection?	Proportion (%) of strains sensitive to test antibiotic among			
		305 strains of *E. coli*	46 strains of coliform bacilli excluding strains of *E. coli*	57 strains of *Proteus*	60 unidentified non-lactose-fermenting strains
Penicillin	No	91	80	95	70
	Yes	74	73	80	60
Sulphonamide	No	76	73	77	70
	Yes	66	78	51	65
Tetracycline	No	91	80	22	88
	Yes	74	86	14	72
Ampicillin	No	95	73	95	70
	Yes	76	69	74	67
Streptomycin	No	89	93	86	82
	Yes	77	95	85	86

among patients with recent urinary infection is most marked with strains of *E. coli* and *Proteus*; and quite distinct with the unidentified non-lactose-fermenting strains, is less clear with the strains of *Klebsiella* and unidentified coliform bacilli, but this may be due in part to the small number of strains (15) in the group with no history of recent urinary infection.

Association of resistance to two antibiotics

Table 28 relates the penicillin resistance of strains of *E. coli* from

patients with urinary tract infection in hospital and in the community with their resistance to eight other antibiotics.

The strains from each source were unselected (as described earlier) and were divided into penicillin-resistant and penicillin-sensitive groups. The proportion of each group resistant to each of the other antibiotics was then calculated and the relation between the two calculated as a ratio.

The results show a striking association between ampicillin and penicillin resistance as would be expected. Thus among the hospital strains ampicillin resistance was 66 times more likely to be associated with penicillin resistance than with penicillin sensitivity. Less marked associations are evident between penicillin resistance and resistance to tetracycline, streptomycin and sulphonamide, and these associations are borne out in both groups.

The results also indicate that an adequate number of resistant strains are essential before meaningful results can be obtained. The association probably spurious, of nitrofurantoin resistance with penicillin resistance in the strains from patients in the community illustrates the quality of the result that may be expected when only 2 - 3 per cent. of all strains are resistant to either antibiotic.

In order to investigate the association of resistance between one drug and the eight other drugs in a single species from a single source required the calculation of 16 sensitivity percentages and a further eight ratios (the latter being done manually in this investigation). To investigate a possible association of resistance between every pair of antibiotics from each species from each source would clearly be impossible without the aid of the computer. However these calculations could be made very rapidly and the results printed quickly in a form similar to the first two columns of

TABLE 28

Penicillin/antibiotic resistance correlation.

The proportion of penicillin-resistant strains of E. coli that were resistant to each of 8 antibiotics compared with the proportion of penicillin-sensitive strains of E. coli resistant to the same antibiotics

Antibiotic tested	Percentage of 100 strains from patients in hospital that were resistant to the test antibiotic among			Percentage of 305 strains from patients in the community that were resistant to the test antibiotic among		
	24 penicillin-resistant strains* 'R'	76 penicillin-sensitive strains* 'S'	Ratio R/S**	58 penicillin-resistant strains* 'R'	247 penicillin-sensitive strains* 'S'	Ratio R/S**
Nalidixic acid	0	1	...	7	1	7
Ampicillin	66	1	66	80	1	80
Nitrofurantoin	4	3	1.3	7	1	7
Kanamycin	0	0	...	10	2	5
Colomycin	12	4	3	2	7	0.3
Tetracycline	54	10	5.4	45	12	3.7
Streptomycin	54	8	6.7	55	10	5.5
Sulphonamide	66	21	3.1	70	10	7.0

* The figure in these columns have been calculated by the computer and "rounded off" to the nearest whole digit.

** Ratio R/S is the ratio of the penicillin-resistant test-antibiotic-resistant percentage over the penicillin-sensitive test-antibiotic-resistant percentage.

TABLE 29

The proportion of ampicillin-resistant, penicillin-resistant strains of different species and groups of species from various sources compared with the proportion of ampicillin-sensitive, penicillin-resistant strains in each group

Description of the strains analysed	Number and percentage of strains resistant to penicillin among				Ratio* R/S
	ampicillin-resistant strains		ampicillin-sensitive strains		
	Number	% resistant to penicillin 'R'	Number	% resistant to penicillin 'S'	
100 strains of *E. coli* from patients in hospital	17	94	83	10	9.4
305 strains of *E. coli* from patients in the community	49	94	256	4	22
57 strains of *Proteus* from patients in the community	10	80	47	0	∞
23 strains of coliform bacilli (excluding strains of *E. coli*) from hospital patients	15	93	8	37	2.5
46 strains of coliform bacilli (excluding strains of *E. coli*) from patients in the community	14	71	32	3	24
60 unidentified non-lactose-fermenting strains from patients in the community	19	84	41	15	18

* Ratio of ampicillin-resistant penicillin-resistant percentage over the ampicillin-sensitive penicillin-resistant percentage.

Table 28. Clearly it is impossible to reproduce all the results even if this were desirable, and so it is necessary to make the selection in order to demonstrate associations that exist and to indicate what the basic requirements are for this kind of work to be successfully pursued. The results from Table 28 indicate that the association between penicillin and ampicillin might be investigated as a control, but also that there may be associations between penicillin and tetracycline, streptomycin and sulphonamide.

Table 29 confirms the relationship between penicillin and ampicillin, but it demonstrates (with the strains of E. coli) that the ratio will depend on which resistance is selected first (compare these results with the results with the same strains in Table 28). It also illustrates the wide scatter that may occur especially when the number of strains is small. Because very few of the strains from patients with bacteriuria of pregnancy or from the faeces of healthy adults were resistant to any antibiotics these groups have been omitted from all the tables demonstrating the association of antibiotic resistance.

Legend for Table 30

The strains analysed in the table were as follows:

1. 100 strains of E. coli from patients in hospital.
2. 305 strains of E. coli from patients under the care of General practitioners.
3. 46 strains of coliform bacilli (excluding strains of E. coli from patients under the care of general practitioners.
4. 57 strains of Proteus from patients under the care of general practitioners.
5. 60 unidentified strains of bacilli none of which fermented lactose, all coming from patients under the care of general practitioners.

TABLE 30

Association of antibiotic resistance

Strains analysed. See legend	Antibiotic B	The ratio of the proportion (%) of antibioticA resistant strains which were also resistant to antibiotic B over the proportion (%) of anti-biotic A sensitive strains which were resistant to antibiotic B when antibiotic A was		
		sulphonamide	tetracycline	streptomycin
1	Tetracycline	7.1	...	5.2
2		4.5	...	10.8
3		1.4	...	1.25
4		1.7	...	1.25
5		1.1	...	0.92
6		1.0	...	0.46
1	Streptomycin	infinity	11	...
2		17	12.4	...
3		2.3	9.3	...
4		4.5	infinity	...
5		1.7	1.1	...
6		1.0	1.5	...
1	Sulphonamide	...	4.7	7.1
2		...	3.5	34
3		...	1.2	6.7
4		...	1.3	2.3
5		...	1.1	1.4
6		...	1.0	1.7
1	Penicillin	4.2	5.5	2.9
2		7.2	3.7	9.5
3		2.7	6.1	25
4		infinity	3.3	0.86
5		3.5	0.72	1.3
6		0.7	0.67	0.86

6. 30 strains of Streptococcus faecalis isolated from patients under the care of general practitioners.

Strains of Proteus and Streptococcus faecalis

Examination of Table 30 shows that (without fixing arbitrary criteria of significance) there is no association of resistance among the strains of Streptococcus faecalis nor among the unidentified non-lactose fermenting strains. Among strains of Proteus streptomycin resistance was only found among tetracycline-resistant strains, and penicillin resistance was likewise found only among sulphonamide-resistant strains. In neither case were the reciprocal associations equally exclusive, the ratios being 1.25 and 3.5 respectively (the latter ratio does not appear in the table). It would seem therefore that small numbers of penicillin and streptomycin-resistant strains (8 strains in each case) and the relatively large number of sulphonamide and tetracycline-resistant strains (22 and 47 strains respectively) caused the apparent relationship.

Strains of E. coli

Among the strains of E. coli from hospital and community sources the evidence for an association of resistance of two antibiotics was greatest between streptomycin and sulphonamide the four ratios being infinity, 34, 17 and 7.1. There was also evidence of an association between streptomycin and tetracycline, the ratios being 5.2, 10.8, 11 and 12.4, and a lesser degree of association between tetracycline and sulphonamide (4.7, 3.5, 7.1 and 4.5). There was, moreover, some evidence of an association between the resistance of each of these drugs and penicillin, the lowest ratio being 2.9 and the highest 9.5.

There is little evidence of any pairing of resistance among strains of

other coliform bacilli. The small number of resistant strains in this group make occasional high ratios difficult to interpret.

Value of the study of the association of antibiotic resistance

The value of this study lies not in the once-only demonstration of possible associations which can be followed up and resistance factors perhaps identified, but in the development of a technique for the rapid estimation of such ratios which can be computed at regular intervals in order to detect at an early stage any general increase in resistance to a group of drugs.

Penicillinase production and penicillin sensitivity

Table 31 records the amount of penicillinase produced by eleven strains of Gram-negative bacteria, and lists also the mean inhibitory concentration of penicillin of the same strains. The test used is a crude one and does not give a clear indication of the rate of penicillinase production, but it does show that even quite small amounts of penicillinase were not detected in the cultures of the penicillin-sensitive organisms, whereas quite large amounts could be (but were not necessarily) produced by the resistant strains. Whilst there was no correlation between the amount of penicillinase produced (as measured by this test) and the penicillin sensitivity of the resistant strains there was a definite distinction between those strains which did produce penicillinase, all of which were resistant, and the sensitive strains which did not produce the enzyme.

Further experiments with strain no. 26 (*E. coli*), however, indicated that some penicillinase was produced by this penicillin-sensitive strain if the culture was incubated for long enough, and that there was bound penicillinase even in an overnight culture.

TABLE 31

Penicillinase production and penicillin sensitivity of strains of E. coli and Klebsiella

Species of bacteria	Number of strain	Penicillin sensitivity (µg per ml) by tube-dilution	Penicillinase production (µg of penicillin destroyed per ml of culture filtrate)
E. coli	26	25	<2.5
	31	25	<0.1
	73	25	<2.5
	40	50	<0.1
	56	50	<2.5
	44	500	100
	35	1000	10
	38	5000	1000
Klebsiella	118	5000	10
	508	2500	100
	43	5000	1000

In the first experiment an overnight broth culture of strain no. 26 was divided. One aliquot was filtered in the usual way, whilst the other was first subjected to 5 minutes treatment in an M.S.E. 100 watt ultrasonicator. No penicillinase (less than the equivalent of 2.5 µg of penicillin destroyed per ml) was detected in the first sample, but in the ultrasonicated sample there was enough penicillinase to inactivate 25 µg of penicillin per ml in the test.

The same strain was then grown in broth and incubated for a week before filtering and testing for penicillinase, which testing yielded the equivalent of 10 µgs of penicillin per ml. These results are summarised in Table 32.

TABLE 32

Penicillinase production in strain no.26

Conditions of experiment	Penicillinase production (µg of penicillin destroyed per ml of culture filtrate)
Overnight broth, untreated	<2.5
Overnight broth, ultra-sonicated	25
Broth incubated for 1 week	10

One-step mutants

All the stored cultures (8 penicillin sensitive and 7 penicillin resistant stored on a shelf and the same number stored in the refrigerator) were tested within two weeks of inoculation and then at intervals during the next 12 months.

In no case was there a significant change in the penicillin sensitivity of any strain. On all occasions when the strains were tested it was noted that a few of the strains from some of the cultures (notably strains 31, 34, and 40) showed a slight increase in resistance. Several of these strains were subcultured distal to the ditch and the penicillin sensitivity measured by tube-dilution. Representative examples are shown in Table 33. No significant increase in penicillin resistance was noted.

TABLE 33

Penicillin sensitivity of strains isolated from the ditch plates

No of strain	Discription	Penicillin sensitivity (µg per ml) by tube-dilution
31	Control (fully sensitive)	25
	Increased resistance indicated by ditch plate	50
34	Control (fully sensitive)	25
	Increased resistance indicated by ditch plate	50
40	Control (fully sensitive) (smooth)	50
	Rough variant	50
	Increased resistance indicated by ditch plate	50
35	Resistant control	1000

The shelf culture of strain 40 changed after 4 months storage from giving uniformly smooth colonies to a mixture of approximately equal numbers of smooth and rough colonies. There was no difference between the penicillin sensitivity of the two variants.

Development of resistance to penicillin

The results of repeated culture of strains of E. coli in gradually increasing concentrations of penicillin are tabulated in Table 34. These show that it was possible to increase the resistance to penicillin of both initially resistant and initially sensitive strains of E. coli

TABLE 34

Development of resistance to penicillin

Steps	Mean inhibitory concentration at each step of the various strains (µg per ml)					
	No. 35	No. 44	No. 4	No. 31	No. 40R*	No. 40S*
1	2500	1000	1000	25	50	50
2	2500	1000	2500	25	50	50
3	2500	1000	2500	25	50	50
4	2500	2500	2500	50	50	50
5	2500	2500	2500	100	100	100
6	5000	2500	5000	100	100	100
7	5000	2500	5000	250	100	100
8	5000	2500	5000	500	100	100
9	5000	2500	5000	500	100	100
10	5000	2500	5000	500	250	100
11	5000	2500	...	500	250	250
12	5000	5000	...	500	250	250
13	5000	5000	...	1000	500	250
14	5000	5000	...	1000	500	250
15	5000	10,000	...	2500	500	250
16	5000	10,000	...	5000	500	250
17	5000	10,000	...	5000	500	250

*40R Strain no. 40 rough.

40S Strain no. 40 smooth.

by 2 - 10 fold in the course of 17 subcultures in increasing concentrations of penicillin, and that the penicillin resistance of strain no. 31 was increased by 200 fold. It is perhaps noteworthy that the degree of penicillin resistance of strain no. 31 increased in two phases from 25 to 500 µg of penicillin per ml over 5 'steps', and then following 4 'steps' with no change in the penicillin resistance a further tenfold increase occurred

to 5000 µg per ml over four 'steps'. In none of the other strains was a similar phasing noted even of their more moderate increases in resistance, and so it may be that the phasing was co-incidental, or it may indicate that underlying enzymatic or biochemical changes or selections were taking place, such as gaining the ability to produce free penicillinase.

Biochemical and serological identity of original and trained sub-strains

The original 'O' strains and trained 'T' sub-strain of strain no's 35, 44, 31, 40R and 40S (strain no. 4 having been lost) were tested with 22 biochemical tests, and in every case the reaction of each pair was identical both quantitatively and qualitatively. The biochemical reactions of the 'O' and 'T' sub-strains of no. 31 are shown in Table 35. They were also serotyped, and both were of the same serological group, '06', but absorption tests were not carried out.

Stability of the change in sensitivity

Following the end of "training" the strains were subcultured 25 times on penicillin free blood agar, and thereafter on to a Dorset's egg slope for storage at room temperature. After 22 months of storage the strains were subcultured once more. The stability of the change in resistance was investigated by measuring the penicillin sensitivity, after 20 subcultures and again after 22 months in storage. The results in Table 36 show that the increased resistance was quite stable, following a considerable time on penicillin-free media. Such differences that there are within the limits of the accuracy of the experiment. The trained sub-strain no. 31 remained 100 times more resistant to penicillin than the original.

TABLE 35

Biochemical identity of the original and trained sub-strains of E. coli strain no. 31

Biochemical test	Result in	
	original strain	trained strain
Fermentation for 24 hours of:		
1% adonitol	-	-
1% arabinose	-	-
1% dextrose	A	A
1% dulcitol	A	A
1% galactose	AG	AG
1% glucose	AG	AG
1% inositol	-	-
1% lactose	AG	AG
1% manitol	AG	AG
1% raffinose	slight A	slight A
1% rhamnose	-	-
1% salicin	-	-
1% sucrose	AG	AG
1% xylose	A	A
Production of indole	+	+
Production of hydrogen sulphide	-	-
Production of urease	-	-
Production of nitrate	+	+
Growth in Koser's citrate medium	-	-
Liquifaction of gelatin	-	-
Methyl-red test	+	+
Voges-Prauskauer test	-	-

A = production of acid G = production of Gas

+ = positive reaction - = negative reaction

The stability of penicillin resistance following 22 months storage did not follow any definite pattern. No strain changed from the 'resistant' to the 'sensitive' category, or vice versa, but there were nevertheless considerable changes in penicillin sensitivity, not only in the trained sub-strains, but also in the original strains. Thus, while one of the two trained sub-strains of no. 31 remained very resistant to penicillin, the resistance of the other was reduced five fold, as was the resistance of the original strain of no. 35, the trained sub-strains remaining unaltered. There was no significant change in the sensitivity of strains 40S or 40R.

TABLE 36

Stability of penicillin resistance

Number of strain	Strain tested before or after storage for 22 months	Penicillin sensitivity (μg per ml) of		
		original strain	trained sub-strain	trained strain after 20 sub-cultures
35	Before	2500	5000	10,000
	After	500	5000	5000
44	Before	1000	10,000	5000
	After	10,000	10,000	10,000
31	Before	25	5000	2500
	After	25	10,000	500
40S	Before	50	250	250
	After	25	100	100
40R	Before	50	500	500
	After	50	500	500

Free penicillinase production of original and trained sub-strains

The free penicillinase production of the original strains, the trained

sub-strains and the trained sub-strains after 20 serial subcultures on penicillin free media was tested. The tests on the original and trained strains were done at the same time, the third test being carried out at a later date after the subcultures in penicillin free medium. The results are recorded in Table 37.

TABLE 37

Free penicillinase production of original and trained strains

No. of strain	Free penicillinase production (equivalent to µg of penicillin destroyed per ml of culture filtrate) of		
	original strain	trained sub-strain	trained sub-strain after 20 subcultures
35	10	10	100
44	100	100	100
31	<0.1	10	100
40S	<0.1	<0.1	<0.1
40R	<0.1	1	<0.1

The most notable change in free penicillinase production occurred in strain no. 31, the original strain of which did not produce detectable penicillinase, whereas the trained sub-strain produced enough to destroy 10 µg of penicillin per ml of culture filtrate. Strain 40R also changed from being a non-penicillinase producing strain and acquired the ability to produce and liberate small quantities of the enzyme, corresponding with an increase in penicillin resistance to an m.i.c. of 500 µgs of penicillin per ml. However after 20 subcultures on penicillin free medium it apparently

lost this ability along with a fall in resistance to an m.i.c. of 250 µg of penicillin per ml. Strain 40S remained a non-penicillinase-producer throughout.

The increase in penicillinase production of strains 35 and 31 during subculture on penicillin-free medium may be due to inaccuracies in the test (which is subject to some variation) due to the fact that the tests were done on different days, or it could be due to an actual increase in penicillinase production. The causes of this change were not investigated, but it may be worth noting that the controls detailed on page 92 were carried out and were correct.

TABLE 38

The effect of adding an equal quantity of sterile urine containing penicillin G to an overnight culture of a strain of E. coli in urine

Time (hr) after the addition of urine with penicillin	Viable count in each of four aliquots of an overnight culture to which penicillin in sterile urine had been added, and in which the concentration of penicillin (µg per ml) in the diluted culture was			
	50	100	250	500
0	4.24×10^8	4.24×10^8	4.24×10^8	4.24×10^8
½	5.20×10^7	3.84×10^7	2.96×10^7	7.20×10^6
2	1.21×10^8	1.40×10^7	6.73×10^5	1.51×10^6
4	...	4.80×10^6	9.68×10^6	8.70×10^6
6	...	5.20×10^8	3.20×10^7	6.50×10^7

Model Bladder

The results of the control experiment in which sterile aliquots of urine containing penicillin were added to equal volumes of an overnight culture of *E. coli* in urine are shown in Table 38. Even when the concentration of penicillin in the culture was 500 µg per ml, the drop in the viable count was only slight and temporary.

Concentrations of penicillin in the model bladder

The theoretical concentrations of penicillin in the model bladder, when the residual volume was 1 ml, 10 ml and 100 ml, are shown in Figure 5. In order to demonstrate the accumulation of penicillin in the bladder when the residual volume was large, three consecutive cycles of penicillin have been illustrated, corresponding to three 'doses' of penicillin. Bladder voiding was synchronised with the theoretical time of administration of the penicillin.

It can be seen that the concentration of penicillin rose rapidly in the first hour to reach a peak in the second and third hour, and was maintained thereafter well above the mean inhibitory concentration of penicillin of most strains of *E. coli* and *Proteus*. When the residual volume was only 1 ml the concentration of penicillin in the model fluctuated widely from a peak of 547 µg per ml, to a minimum of 63 µg per ml soon after voiding. When the residual volume was large (100 ml) there was a significant degree of accumulation of penicillin so that the peak concentration after the second dose (370 µg per ml) was one third higher than the peak after the first dose (285 µg per ml). After the third dose, however, there was little further accumulation so that the concentration thereafter ranged from a peak of

almost 400 µg to a minimum soon after voiding of 220 µg per ml.

Effect of dilution with out penicillin on the concentration of bacteria in the bladder

The result of an experiment in which an infected residual volume of 1 ml was diluted in the model bladder is shown in Figure 6. After 8 hours the number of bacilli per ml was similar to the number at the beginning of the experiment.

The calculated effect of diluting infected residual urine assuming that there was complete inhibition of growth, but no lysis of bacilli is also shown in Figure 6. When the residual volume was 1 ml there was a steep decline in the concentration of bacilli per ml during the first hour, followed by a decreasing reduction in the concentration every hour until the 6th hour when the bladder was voided, after which a further steep decline in the number of organisms per ml began. When the residual volume was 10 ml, the effect of complete inhibition of growth on the concentration of organisms per ml was less - as expected. With a residual volume of 100 ml, there was very little effect, and this has not been illustrated.

Experiments with the model bladder

The results of the experiments with the model bladder are shown in Figure 6. Two strains of E. coli were tested. One was inhibited by 25 µg of penicillin G per ml, and it did not produce detectable free penicillinase. The other strain required not less than 1000 µg of penicillin G for inhibition, and was shown to produce enough penicillinase to destroy 100 µg of penicillin per ml of culture filtrate.

Results with the penicillin sensitive strain of E. coli

This strain was tested thrice in the model bladder with residual volumes of infected urine of 10, 50 and 100 ml. In each case the culture was sterile

FIGURE 5

The concentration of penicillin in the model bladder following the addition of aliquots of fresh urine containing penicillin to residual infected volumes of 1, 10 and 100 ml.

Three cycles of the administration of the penicillin containing aliquots of urine are illustrated, equivalent to three 'doses' of penicillin. The bladder was voided every 6th hour.

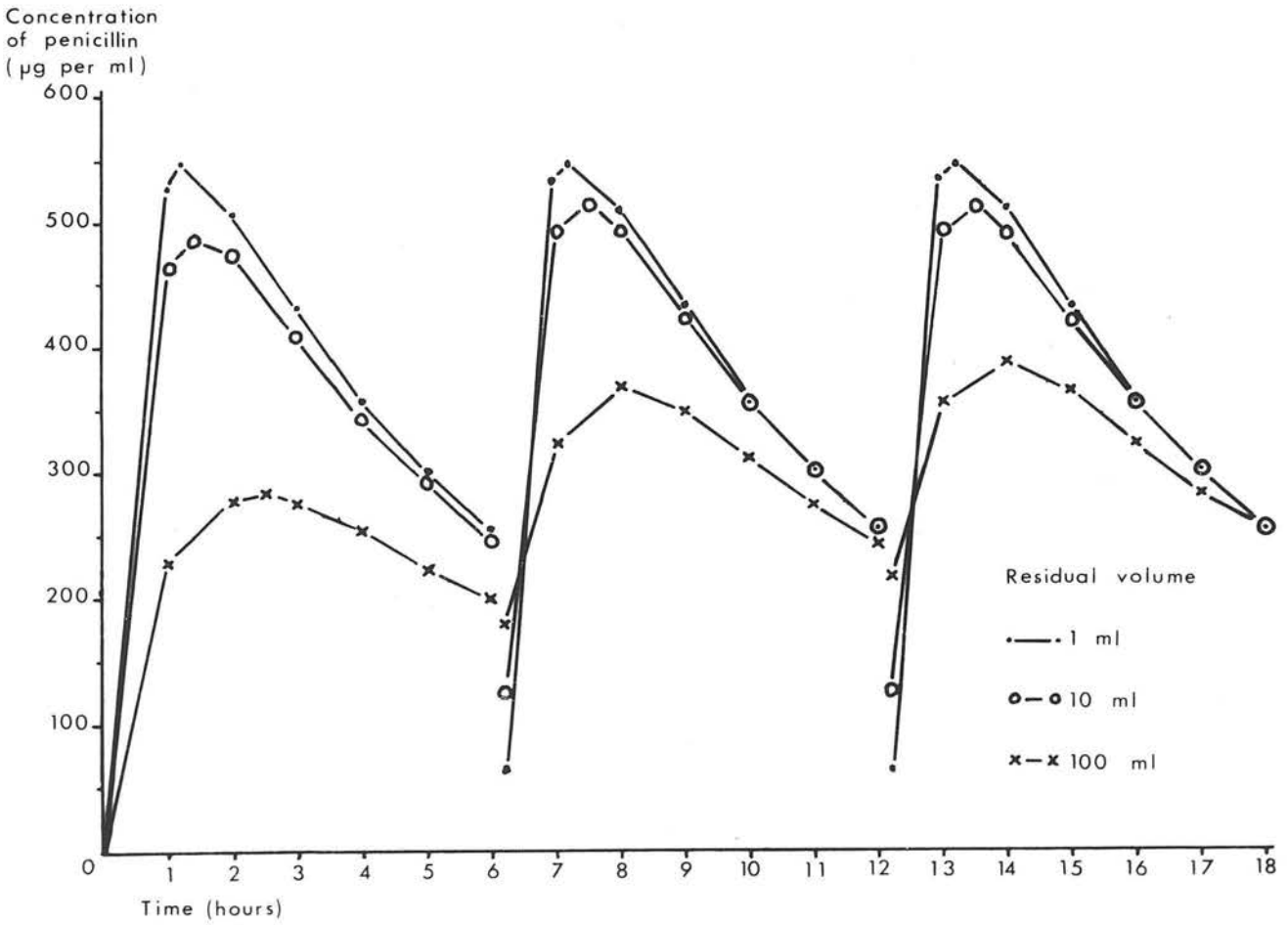
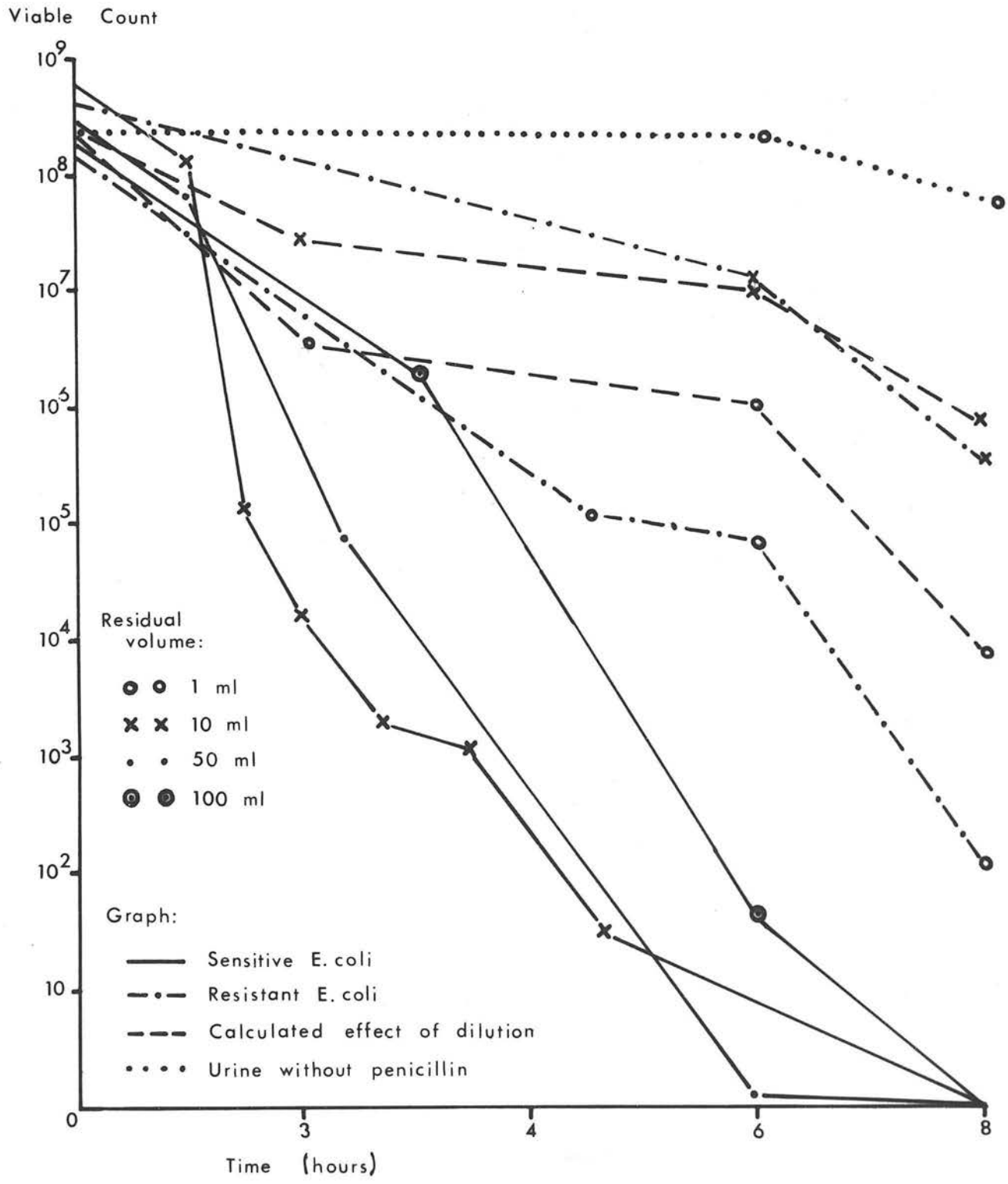

Concentration
of penicillin
(µg per ml)
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Time (hours)
Residual volume
1 ml
10 ml
100 ml

FIGURE 6

Results of experiments with the model bladder

Illustrated in this figure are:

1. The effect on the viable count per ml of diluting an infected residual volume of 1 ml with sterile ureteric urine without penicillin (dotted line). There was no significant effect.
2. The calculated effect on the viable count (per ml) of dilution, assuming that there is neither multiplication or lysis of bacilli (dashed line). This is calculated for residual volumes of 1 and 10 ml.
3. The results when the residual volume was infected with a penicillin resistant strain of E. coli (dash-dot line). When the residual volume was 10 ml, the effect on the viable count per ml was similar to that calculated to occur with bacteriostasis, but with a residual volume of only 1 ml there was bacterioslysis.
4. Results when the residual volume (of 10, 50 and 100 ml) was infected with a penicillin-sensitive strain of E. coli (continuous line). Rapid bacteriolysis is demonstrated, with a sterile urine being achieved by the 8th hour.


Viable Count
10^9
10^8
10^7
10^5
10^5
10^4
10^3
10^2
10
0
Residual volume:
1 ml
10 ml
50 ml
100 ml
Graph:
Sensitive E. coli
Resistant E. coli
Calculated effect of dilution
Urine without penicillin
3
4
6
8
Time (hours)

(less than 8 bacilli per ml) by the 8th hour, after beginning the second 'dose' of penicillin. The quantity of residual urine did not affect the slope of the graph (indicating the rate of the elimination of the organisms), but when the residual volume was large, the reduction in the viable count was delayed.

During the experiment 37 colonies from the blood agar plates used when performing viable counts during the 6th hour of dilution in the bladder, were subcultured, and the penicillin sensitivities of 7 tested by tube-dilution, and of 30 by disk-diffusion. None showed a significant change in sensitivity.

Results with a penicillin-resistant strain of E. coli

One and 10 ml volumes of residual urine infected with this strain of E. coli were tested in the model bladder. At no time during the cycle of penicillin excretion by the model did the concentration of penicillin in the model reach the concentration required for inhibition of this strain by conventional tube-dilution techniques (see Figure 5). Nevertheless, when the residual volume was 10 ml, the reduction in the viable count was approximately equal to that which would have occurred had there been complete bacteriostasis but no bacteriolysis. When the residual volume was only 1 ml the reduction in the viable count was much greater than could be accounted for by bacteriostasis, and indicated that lysis of the bacilli had taken place.

Although no measurements of turbidity were made when the residual volume was 1 ml the clearing of the culture corresponding to the lysis of the bacilli was readily perceptible to the naked eye. Up to 3 - 3½ hours after the experiment began there did not appear to be any reduction in turbidity

compared with that of a fully grown culture, but during the fourth and fifth hours clearing took place until no turbidity could be detected by the eighth hour.

DISCUSSION

Introduction

The theme of this section has been to correlate the penicillin sensitivity of the Gram-negative bacilli with the concentration of penicillin that is readily attainable in the urine after oral therapy with moderate doses of penicillin.

Briefly, it has been demonstrated that most Gram-negative bacilli are inhibited by concentration of penicillin that can be maintained in the urine for 4 -6 hours after an oral dose of potassium penicillin G. Moreover, experiments with an in-vitro bladder have suggested that in a continuous dilution system the sensitivity of strains of E. coli may be greatly enhanced.

Penicillin concentrations in urine after oral therapy

There can be no further doubt about the concentration of penicillin that may be achieved in the urine after oral administration of Potassium penicillin G. The measured concentration in the urine excreted during the first two hours after the drug was administered was not less than 512 µg per ml in any of the subjects studied. This level declined rapidly so that the mean measured concentration of penicillin in the urine excreted in the 5th and 6th hours was not above the minimum required to inhibit all sensitive strains of E. coli. Should the concentration of penicillin in the urine temporarily fall below this minimum, effective treatment is unlikely to be prejudiced, as Eagle (1949) noted that bacteria take some time to recover from the effects of exposure to penicillin before multiplication and a return to virulence occurs, and that during this time those bacteria that have survived the penicillin are extraordinarily sensitive to the normal defence mechanisms of the body. In

the bladder therapeutic concentrations of penicillin can be maintained by timing micturition to coincide with the administration of the antibiotic (and this arrangement has other advantages which are mentioned later), but in the medulla of the kidney the existence of this recovery period is an important factor in deciding whether a six-hourly dosage scheme is acceptable or whether a four-hourly regimen ought to be adopted. For the clinical trial which is reported later in this thesis penicillin was given six-hourly.

The very high initial concentrations of penicillin in the urine are unlikely to exert any greater effect on penicillin-sensitive pathogens than the optimal concentrations (Eagle, 1951), but they do allow a considerable margin of therapeutic efficiency so that even in the presence of large quantities of residual urine the mean inhibitory concentration of the infecting organism may still be exceeded, and even if a patient does not absorb penicillin well the urine concentrations in the bladder are unlikely to be so low as to be ineffective. The subject (AB) with the poorest excretion of potassium penicillin G reported in this thesis had, nevertheless, a mean penicillin concentration of 150 µg per ml of the urine excreted during the first 6 hours after an oral dose 500 mg of potassium penicillin G.

When the penicillin concentration in the urine of patients being treated with oral potassium penicillin G for urinary infection was measured, the results indicated that the existence of infection did not alter the pattern of the excretion of the drug.

Some general observations made by earlier workers have been confirmed by the results of the present study, but some of the findings were unexpected. Thus the mean measured excretion of penicillin G (13.4 per cent. of the dose) was within the range of that recorded by McDermott et al. (1946), and similar

to the proportion reported by Friend (1966), and the serum concentrations of penicillin were also within the range reported by other workers (see Table 1). On the other hand, the results of the administration of penicillin post-prandially did not confirm the observation of Finland and his colleagues (1945) that while pre-prandial administration resulted in good and regular absorption with predictable serum levels, this was not the case following meals. The results of the very limited experiments reported in this thesis (which did not include the measurement either of the serum levels nor the actual amount of penicillin excreted) did suggest that post-prandial administration delays peak urine concentrations of penicillin, but does not depress them.

A comparison of the pattern of urinary excretion following parenteral administration, with that following oral dosage was not made, but McDermott (1946) noted in several investigations that they were very similar and that penicillin absorption was rapid and took place for only a short time after the drug was taken, and Eagle (1947) showed that after a intramuscular injection of penicillin 60 per cent. of the dose appeared in the urine in the first hour. The results of the investigations reported here indicate that oral administration does result in a useful delay in absorption so that the proportion of the total amount of penicillin G excreted in the urine that had been excreted during the first <u>two</u> hours was only 54 per cent.

A further flattening of the peak absorption and excretion of penicillin would lead to more even urine concentrations. It may be that this could be done by taking penicillin with food or post-prandially. While this would lead to a fall in the peak serum concentrations it may not alter the total urinary excretion, and the pattern of excretion might be improved.

Comparison of three preparations of penicillin G

The comparison of the three different preparations of penicillin did not reveal differences between them that were significant for the treatment of urinary tract infection. The concentrations in the urine of each of them was greatly in excess of the mean inhibitory concentration of most Gram-negative bacilli during the first four hours after administration, and the three drugs gave similar (adjusted) levels during the third two hour period.

The sustained action penicillin tablets, marketed for their ability to withstand the action of gastric acid with improved absorption resulting, were, in this experiment, the most poorly absorbed. Moreover far from giving sustained action, a greater proportion of the total excreted in the urine, was excreted in the first two hours (67.5 per cent.), compared with either of the other two preparations (54.5 per cent. for potassium penicillin, and 48.5 per cent. for penamicillin). Even the serum levels of this drug (adjusted to take account of the differing dose) were no higher than those recorded for potassium penicillin 6 hours after administration of the drug, and were lower than those recorded following penamicillin.

Penamicillin was slightly better absorbed than potassium penicillin, and the urine concentrations of penicillin were a little higher during the 5th and 6th hours after the drug was administered. The serum levels of penicillin following a dose of penamicillin were remarkably constant.

These differences, however, are only of marginal significance in the treatment of urinary tract infection, and considering the difference in the price of the drugs (see Materials and Methods, page 79) potassium penicillin G

is the most cost-effective.

The results with the enteric coated tablets confirm those recorded by workers in this field a quarter of a century ago. Abraham et al. (1941) and Rammelkamp and Helm (1943b) both noted that enteric coatings do not improve the absorption of penicillin from the gut. It is possible that they merely protect the penicillin from the action of the gastric acid in order to deliver it to the penicillinase producing organisms in the ileum and colon.

Sensitivity of Gram-negative bacilli to penicillin

Of the 969 strains (935 of them Gram-negative bacilli) isolated from the urine of patients suffering from a urinary tract infection, or from the faeces of healthy adults, 83 per cent. were inhibited either by 50 μg per ml of penicillin in a tube test, or by the comparable disk-diffusion test using a disk containing 100 μg of penicillin. These strains may be said to be sensitive to urinary concentrations of penicillin after oral therapy with a dose of 500 mg six hourly.

The mean inhibitory concentrations of the various strains of bacilli of different species recorded in this investigation confirm those noted by earlier workers of whom Duguid (1946) and Barber et al. (1962) are representative.

Penicillin disks

The disk containing 100 μg of penicillin was found to be satisfactory in subsequent use. Generally there was either a substantial zone around the disk, or growth occurred right up to the margin of the disk, so that, except in a small number of cases there was no difficulty in interpreting the result.

However this disk was the one with the least quantity of penicillin that could fulfil the requirements of zone size (14 mm) with strains of E. coli

which were sensitive to 50 µg of penicillin by tube-dilution tests, and even with this criterion 2 of the 39 sensitive strains tested had zones 1 mm in diameter smaller than the minimum (see Table 21). Furthermore the zone diameters did not equal those obtained with the disk containing 25 µg of ampicillin, being 4 - 6 mm smaller. This ampicillin disk is a standard 'Oxoid' preparation, and is in use in many laboratories. The comparison of penicillin and ampicillin sensitivities of all the isolates showed (Table 25) that 5 - 10 per cent of strains were ampicillin-sensitive but penicillin-resistant. As Sutherland (1964) explains, a proportion of ampicillin-sensitive, penicillin-resistant strains can be expected because of the greater stability of ampicillin to penicillinase. However the comparison of the sensitivity of bacteria to the two antibiotics would have been more valid had the zones produced by the two disks been approximately similar for strains of bacteria sensitive to both drugs.

In retrospect therefore it was regrettable that the 200 µg disk was discarded at such an early stage of the investigation. Further trials of the 200 µg disk might show that it was superior to the 100 µg disk for the purpose of distinguishing those organisms that are sensitive to penicillin and correlating this figure with the proportion sensitive to ampicillin. Conversely, of course, the concentration of ampicillin in the ampicillin disk could be reduced.

Penicillin sensitivity of strains of Gram-negative bacilli from different sources

The varying proportion of strains from the different sources which were sensitive to penicillin demonstrates clearly the increasing resistance to the antibiotic as general exposure to antibiotics and the hospital environment

increases. All strains isolated from patients attending the antenatal clinic were sensitive to penicillin, compared with 91 per cent. of strains from patients in the community without a known history of recent urinary infection, and 74 per cent. of patients in the community with a history of recent urinary infection.

The proportion of urinary strains that were E. coli in the antenatal series was 79 per cent. and this was considerably lower than the proportions reported by Kincaid-Smith (1964) and Turner (1961). Moreover the proportion of urinary strains that were E. coli was even smaller in the general practice group, being only 58 per cent. among those patients with a history of recent urinary infection. Nevertheless this percentage is considerably higher than that recorded by Coleman and Taylor (1949) who found only 18 per cent of patients with a demonstrable lesion in the urinary tract were infected with strains of E. coli.

Penicillin sensitivity and penicillin resistance in Gram-negative bacilli

The results of the tube-dilution sensitivity tests of strains of E. coli, and Klebsiella demonstrate clearly the division of the strains into two groups, those inhibited by 150 µg of penicillin per ml, and those requiring not less than 500 - 1000 µg per ml for inhibition. There were no strains with intermediate sensitivities, in fact the great majority of the penicillin-sensitive strains were inhibited by 50 µg of penicillin per ml, whilst the majority of penicillin-resistant strains required 1000 µg per ml or more for inhibition.

Whereas all the resistant strains tested produced unbound penicillinase, and none of the sensitive strains did so under normal circumstances, the quantity of penicillinase produced, as measured by the crude method employed,

was not in proportion to the degree of penicillin resistance, and did not appear to be the only factor involved in penicillin resistance. These results confirm the finding of Sutherland (1964), but contradict those of Ayliffe (1963). Ayliffe could detect no correlation between the sensitivity or resistance of strains of E. coli to ampicillin and the ability of the strain to destroy ampicillin, whereas strains of Proteus mirabilis did show a close correlation between penicillinase production and ampicillin resistance.

The existance of basic physiological differences between the strains of E. coli that were sensitive to 50 µg of penicillin per ml, and those that required 500 µg or more for inhibition is suggested by the manner in which the penicillin resistance of strain no. 31 increased in two definite stages rather than in a steady progressive manner when it was cultured in sub-lethal doses of penicillin. It seems likely that each stage was associated with the selection of a factor conferring the ability to resist an increased quantity of penicillin. Certainly, as shown by the experiments, this strain acquired the ability to produce penicillinase, although unfortunately the stage at which it did so was not determined.

However strain no. 26, a penicillin-sensitive strain, was shown to produce small amounts of free penicillinase after incubation for 7 days, and also was shown to contain small amounts of bound penicillinase after only 18 hours incubation. It is not certain whether a few bacilli were producing all the penicillinase under these circumstances, or whether all the bacilli were equally involved in producing small amounts of penicillinase. Whichever is the case it seems that some, if not all, penicillin-sensitive strains of E. coli do have an inherent ability to produce penicillinase. It may be therefore that in these strains this ability is merely developed, or concentrated, rather than acquired.

Smith (1963) considered that the penicillinase-producing strain of E. coli that he isolated from a penicillin-sensitive strain while training that strain to penicillin-resistance, was a mutant, and that the mutation that gave rise to it was a rare event. On the other hand Percival et al. (1962) isolated penicillinase producing mutants from penicillin-sensitive strains without difficulty, and also demonstrated small amounts of penicillinase in penicillin-sensitive strains. These findings correspond with the results of the experiments carried out in this section of the thesis in which it has been shown that two out of three penicillin-sensitive strains became, on training, penicillinase producers, although one of the strains (no. 40R) lost this ability, without any reduction in penicillin-sensitivity, after subculture on penicillin-free media.

In the light of these reports from different quarters (Percival et al., 1962; Smith, 1963), and of the results from the experiments reported in this thesis, the development of penicillin-resistant, penicillinase-producing variants of infecting penicillin-sensitive strains of E. coli must be expected in vivo if patients are treated with penicillin or ampicillin. A change of this nature would be difficult to prove, but Stamey and his colleagues (1965) do consider that it occurs among patients treated for Gram-negative urinary tract infection with penicillin. In a proportion of these patients treatment failed due to a change in the sensitivity of the infecting strain which became resistant to penicillin. This occurred only in patients that were treated with penicillin, but it is fair to note that Stamey did not use ampicillin.

The results of the experiment in which penicillin-sensitive and penicillin-resistant strains of E. coli were stored for a year in broth culture and sub-cultured periodically showed that penicillin sensitivity and resistance were

more stable than the penicillin-resistance of strains of Staph. aureus observed by Barber (1949). However, the more precise measurements of penicillin sensitivity made on cultures stored on Dorset's egg medium for 22 months (without passage) shows that considerable changes may occur in penicillin sensitivity within a fairly limited range. Thus although 5 or even 10 fold changes in sensitivity occurred, no strain changed from the 'sensitive' group to the 'resistant' group or vice versa, and there was no significant change at all in the four trained sub-strains that were in the 'intermediate' range, having mean inhibitory concentrations of between 100 and 500 µg per ml.

Computer

The relentless increase in requests for clinical bacteriological investigations that was 50 per cent. over the seven year period from 1958 - 1965, (Data processing in clinical pathology, Journal of Clinical Pathology (1968) vol. 21, page 231), and was 6 per cent. in the diagnostic laboratory of the University of Dundee from 1969 - 1970, makes some kind of automatic data processing desireable if the laboratory is to offer as good a service as was done in the past with fewer specimens and relatively more staff. Because of this, and because of the necessity for medical staff to become involved in the programme production and development, the author was prompted to use the research problems of these investigations as an exercise in bacteriological computing.

By using some other method of analysis, e.g. punched cards, most of the correlations could have been made with the exception perhaps of those relating to the association of resistance to one antibiotic with resistance to another.

Some of the limitations of using a computer for this kind of research

work are obvious. In particular, although 969 strains of bacteria seemed to be a large enough sample, experience proved that when divided up into different species, and again into the various sources, there were not enough strains of any one species except E. coli to make computer analysis really worth while. Also the time and expense (computing time is very expensive) involved in the development of the programme should not be minimised.

However the advantages of using a computer should not go unstressed. By taking the labour out of counting up the numbers of strains in this or that category, any analysis may be performed regularly during the investigation permitting an assessment of the progress of the work, and foreknowledge of what the final results will be, so encouraging modification and development of the investigation. Also, computer results are more precise, more easily checked, and the source of errors which are often illogical and therefore easily detected may be sought and corrected, and the programme re-run without difficulty. This contrasts with the author's experience of other methods where counting errors occur frequently and are difficult to detect, check or trace.

Moreover with the completion of the thesis the fruits of the time and trouble spent developing the programme have not necessarily all been harvested. Parts of it, especially, in this case, those parts relating to the analysis of antibiotic sensitivity tests may be used for other purposes, such as the analysis of results from the routine laboratory for presentation to the clinicians, as O'Brien (1969) advocates.

Linked resistance

A knowledge of the occurrence of linked resistance in a single bacterium, and of the prevalence of strains carrying such groupings, which may be

mediated by resistance transfer factors, is important because clinical failure with a drug previously shown to be active against the infecting organism may be due to the emergence of a resistant strain, insensitive not only to the drug used, but to several other drugs as well. A knowledge of the local prevalence of any linked resistance enables a clinician to select an appropriate second antibiotic for the patient whose initial treatment has failed without waiting for the results of further bacteriological tests. Such groupings will probably not be common among the 'wild' strains of Gram-negative bacilli infecting the patient in the community, but they may be more common, and will tend to become concentrated in the hospital environment. In the future information about these groupings, and a knowledge of their prevalence may become indispensible for the formulation of a hospital antibiotic policy.

Where resistance to a group of drugs has become very common, and is associated with virulent strains of bacteria then it is usually easy to detect, and requires no sophisticated equipment to do so. Nevertheless, in many laboratories, a single bacteriologist has to oversee a very large number of specimens so that even the obvious may be overlooked. The computer, however, could be used to detect such a grouping at a fairly early stage before its presence could be noted clinically, and to monitor its development and spread. Armed with this information logical steps to combat the situation may be taken and it may be kept under regular review in a way which would not be possible by any other means.

The use of the computer to detect antibiotic resistance associations was successfully carried out by O'Brien (1969) who predicted the presence of particular resistance transfer factors, and subsequently demonstrated their

existence by transferring the resistance to a susceptible strain of E. coli.

The technique employed in this study has been different, but it has confirmed the close relationship between ampicillin and penicillin sensitivity (and it might have been closer had the filterpaper disks used for each antibiotic been properly matched, see page 150) and it has also shown significant associations between tetracycline and sulphonamide and tetracycline and streptomycin among strains of E. coli. The importance of this lies not primarily in the research study of resistance transfer factors, but in the everyday clinical problem of making decisions about the most suitable antibiotics for any particular circumstances. A monthly analysis of the antibiotic sensitivities of bacteria isolated from a single hospital could demonstrate (among much other useful data) the emergence of strains resistant to a defined spectrum of antibiotics, and could result in a more informed clinical assault on the problem.

Interchangeability of penicillin and ampicillin

The close relationship between ampicillin and penicillin sensitivity has been discussed above. This is confirmed by the studies with penicillinase which have indicated that those strains which are sensitive to penicillin (and ampicillin) do not produce any free penicillinase, so that the differential stability to penicillinase noted by Sutherland (1964) is not relevant under these clinical circumstances except, perhaps, in a small proportion of strains. It is also clear that although ampicillin is more active weight for weight, at the dosage commonly prescribed for each there is an excess of active drug present in the urine, so that the enhanced absorption of ampicillin, and its greater activity seem to confer little advantage over penicillin in this respect. It is true that ampicillin may achieve tissue concentrations which

exceed the m.i.c. of the infecting organism, but whether or not this is significant, few diagnostic laboratories consider it important enough to report to the clinician, for urinary pathogens are commonly tested against high concentrations of ampicillin only.

If the assumption is made that urine concentrations of antibiotic are significant in urinary infection, then it would appear that penicillin G taken by mouth could be used in place of ampicillin on most if not all occasions.

Model Bladder

The results of the control experiment in which an equal quantity of urine (to provide nutrition and dilute waste products) containing penicillin was added to an overnight culture of Gram-negative bacilli in a static situation should be contrasted with the results of the similar addition of an initially high but decreasing concentration of penicillin under the dynamic conditions existing in the model bladder. In the static experiment the penicillin in a final concentration of 500 µg per ml, produced only a moderate and temporary reduction in the viable count although conventional sensitivity testing indicated that the strain of E. coli was inhibited by 25 µg of penicillin per ml. In the model bladder a concentration of 285 µg of penicillin per ml (which was the peak concentration of penicillin achieved in the first 6 hours in the model bladder when the residual volume was 100 ml) produced a dramatic reduction in the viable count leading to the elimination of the infecting organism. Moreover, in the model, a peak concentration of 547 µg of penicillin per ml caused lysis of a strain of E. coli which was inhibited only by 1000 µg per ml by conventional tube-dilution sensitivity testing.

It is evident that there are many factors operating in the kidney and bladder during antibiotic therapy. Among these are the sensitivity of the organism to the antibiotic used as measured by conventional-tube-dilution or disk-diffusion techniques and the effect of dilution, both of which have already been discussed. Greenwood and O'Grady (1969) have discovered a new factor, or group of factors. They have demonstrated that strains of the same species (Proteus mirabilis) which differ in their ability to swarm, but which have similar or identical sensitivity to ampicillin when tested by conventional methods, have nevertheless contrasting reactions in the model bladder which they used. The fundamental nature of the heterogeneity of the response in the model bladder was confirmed by evidence of a different morphological reaction to ampicillin when the bladder cultures were examined by electron microscopy.

The importance of synchronising micturition and administration of the antibiotic is not frequently emphasized, although it has been mentioned in connection with ampicillin by O'Grady and Pennington (1967). Shortly after the drug has been taken the ureteric urine delivers large quantities of antibiotic into the bladder. If the patient empties his bladder just before this occurs the delivery of the antibiotic will coincide with the period when the residual urine is least, so giving high concentrations of antibiotic in the bladder urine, and also with the period of most rapid dilution of the residual infected urine with antibiotic-containing ureteric urine. The results suggest that these factors may have a cumulative effect, and that together they are rapidly bactericidal.

Where the residual volume is low, frequent micturition, although it will tend to lower the concentration of penicillin in the bladder, will allow the

combined antibiotic-dilution action to take place more frequently. Where there is a large residual volume the effect of dilution is, to a considerable extent, lost, and it is probable that the optimum pattern of micturition would allow accumulation of the antibiotic in the bladder urine. This can best be done if the patient empties the bladder at about the time that the drug is taken, and not oftener. If this were done, theoretically the bladder concentration of penicillin in a patient with a residual volume of 100 ml, who was taking 500 mg of penicillin every six hours, would after three or four doses achieve a minimum concentration of penicillin in the bladder which would be substantially above 50 µg per ml, and perhaps in excess of 200 µg per ml, with peak concentrations considerably greater than this minimum level.

In the medulla the rate of dilution of the infected urine depends only on the rate of excretion of urine which in turn depends on fluid intake. In order to get the maximum dilution effect, and also the most efficient wash-out, fluid intake should be high, recognising that this will be achieved at the expense of the antibiotic concentration which will be lower. The optimum combination of these factors is not known.

The differing roles that antibiotic concentration, dilution, hypo-tonicity of the urine, wash-out, and hitherto un-investigated bacterial factors play, have all to be evaluated. Clearly it is a complex matter. For the present however, it may be wise to attach less importance to the conventional antibiotic sensitivity of a urinary pathogen. While it is shown in this thesis, that those patients with recurrent infections are frequently infected with an antibiotic resistant organism, and that these infections are more rarely treated successfully than primary infections, the resistance of the organism may be nothing more than a measure of the failure of treatment, and may have little to do with the cause of it.

CONCLUSIONS

Unequivocal evidence has been produced to show that the concentration of penicillin in the bladder urine of an adult patient taking 500 mg of potassium penicillin G by mouth every six hours would be greatly in excess of the mean inhibitory concentration of most urinary pathogens if bladder voiding coincided with the administration of the antibiotic, and the concentration of penicillin in the medullary urine would also exceed the m.i.c. of these pathogens for more than 4 hours after each dose.

If the basic assumption, that serum concentrations of antibiotic in excess of m.i.c. of the infecting organism are necessary for treatment, is rejected (and evidence has been cited to support this rejection), then it is concluded that the possibility of effective treatment with oral penicillin G is strong enough to merit a clinical study.

The sensitivity of a urinary pathogen to penicillin or ampicillin as measured by conventional tube-dilution or disk-diffusion tests may underestimate its susceptibility to that drug under the dynamic conditions existing in the urinary tract. Some bacteria appear to be exquisitely sensitive to penicillin when continuous exposure to fresh penicillin excreted by the kidney coincides with the continuous supply of fresh medium (urine) for growth.

The timing of micturition in relation to the administration of the antibiotic is most important. All patients should empty the bladder at the time that the antibiotic is taken. Those with a normal residual volume may benefit from more frequent micturition, those with a large volume are less likely to do so.

The close relation between the sensitivity to ampicillin and to penicillin among Gram-negative bacilli has been demonstrated. In most cases the data

suggests that there will be little advantage to be gained from the use of the more expensive and toxic ampicillin in place of penicillin since at the usual dose of each a considerable excess of drug is present, and there is no evidence that an excess of ampicillin is more effective than an excess of penicillin, or that ampicillin is usually prescribed for patients with urinary tract infection after a consideration of the sensitivity of the organism to the concentrations of ampicillin reached in the tissues.

The occasional development of resistance to penicillin and ampicillin by infecting organisms in vivo is to be expected. Within a scarred or cystic kidney, or in the interstices of a staghorn calculus there are certain to be situations where only sub-lethal doses of penicillin penetrate, and where an infecting strain of E. coli may develop permanent, penicillinase-producing resistance.

Computers may be employed to provide clinicians with useful additional bacteriological information which would not be available otherwise. In particular information about the overall sensitivity of pathogens to various antibiotics, andabout the emergence of linked resistance, possibly associated with resistance transfer factors could be provided in addition to the normal service.

SUMMARY

1. The urinary excretion of three oral preparations of penicillin G was measured in a cross-over trial in six subjects. Mean urinary concentrations of over 500 µg per ml were achieved in the first 2 hours after a dose of 500 mg of crystalline potassium penicillin G, dropping to 40 µg per ml between the fourth and sixth hours after the dose.

The differences in the excretion of the three preparations of penicillin G tested (potassium penicillin G, an acetoxymethyl ester of penicillin which is hydrolysed to penicillin in the body, and an enteric coated preparation) were not important therapeutically, but there were substantial differences in cost. The urinary excretion of potassium penicillin G was 13.4 per cent. of the dose.

2. Various filter-paper disks containing different amounts of antibiotic were tested, and compared with tube-dilution tests. A disk containing 100 µg of penicillin was considered to give results which correlated well with a tube-dilution sensitivity of 50 µg per ml. This disk was then manufactured commercially.

3. The penicillin sensitivities of 793 urinary pathogens (including 758 Gram-negative bacilli), and of a further 176 Gram-negative bacilli isolated from the faeces of healthy adults, were determined. Eighty three per cent. were sensitive either to the filter-paper disk or to 50 µg of penicillin per ml by tube-dilution.

4. Strains of E. coli were found to fall into two groups: those sensitive to 50 µg of penicillin per ml, and these did not produce free penicillinase

under normal conditions; and those strains requiring at least 500 µg, and some requiring 10,000 µg of penicillin per ml for inhibition and these did produce free penicillinase. The sensitive group comprised between 76 and 100 per cent. of strains of E. coli depending on the source of the strains.

5. The strains of Gram-negative bacilli were grouped according to the probability of the patient (or subject) having had recent exposure to antibiotics or the hospital environment. As the probability of this exposure increased, so the proportion of penicillin-sensitive strains decreased, and the proportion of all strains that were E. coli also decreased.

6. The sensitivity of 857 of the strains to 8 antibiotics was tested by disk-diffusion. A computer was used to investigate links between the antibiotic resistance of different drugs. The relationship between penicillin and ampicillin was investigated as a control, and subsequently evidence of an association between resistance to streptomycin and tetracycline, and between resistance to tetracycline and sulphonamide among strains of E. coli was presented.

7. The stability of penicillin sensitivity and resistance was investigated by two experiments previously performed with strains of Staphylococcus aureus. In the first penicillin-sensitive and resistant strains stored for a year were quite stable, whereas Barber (1949) found that penicillin-resistant strains of Staph. aureus stored under similar condition gave off sensitive mutants. In the second experiment the m.i.c. of one of three penicillin sensitive strains of E. coli increased from 25 µg per ml in two stages to

5000 µg per ml when it was cultured in sub-lethal concentrations of penicillin. This change was stable over many subcultures in penicillin free media, and after storage, and it was associated with the acquisition of the ability to produce free penicillinase.

8. A model bladder was constructed containing a quantity of 'residual infected urine' to which warmed sterile urine was added at a rate of 15 ml every 15 minutes. Penicillin was added to each aliquot to give approximately the concentrations that were found in the urine of the six subjects at the appropriate time after an oral dose of 500 mg of penicillin G. A strain of E. coli sensitive to 50 µg of penicillin per ml was eliminated from this model within eight hours, even when the quantity of residual urine was 100 ml, and the viable count in the residual urine was over 10^8 bacilli per ml. When a strain of E. coli which was inhibited by not less than 1000 µg of penicillin per ml was tested, bacteriolysis was demonstrated when the residual volume was 1 ml, and bacteriostasis when the residual volume was 10 ml.

SECTION 11

The laboratory diagnosis of infections of the urinary tract of patients whose specimens of urine have to be sent by post

INTRODUCTION

The purpose of this part of the work, is to evaluate the dip-inoculum spoon first reported by Mackey and Sandys in 1965, and to explain the finding that Gram-positive cocci gave fewer colonies per spoon for the same viable count than Gram-negative bacilli. A programme of testing the spoons in the laboratory was carried out, and the spoons were then used in a hospital clinic, and later by general practitioners in the course of a trial of the treatment of urinary tract infection with penicillin G. In the course of the testing some small modifications were made to the outfit, and a different method of inoculating the spoons was developed.

MATERIALS AND METHODS

The dip-inoculum outfit

The spoons used by Mackey and Sandys were of varnished metal. A few experiments were carried out with similar spoons made in the laboratory, but difficulties were encountered in preserving the varnish film intact, and the spoons used were fragile, not standard in size, and clearly unsuitable for large-scale use. In 1966 an autoclavable plastic spoon became available (supplied by the Medical Wire and Equipment Co. (Bath) Ltd), and these have been used exclusively for the experiments reported below.

However the plastic spoons caused an unexpected difficulty. The Cystein, Lactose, Electrolyte Deficient (CLED) medium used by Mackey and Sandys is a blue colour, lactose fermenting colonies appear yellow upon it, and non-lactose fermenting colonies are a pale grey. The spoons are green, and the combination of these colours makes the identification and accurate counting of the colonies impossible. After carrying out some experiments MacConkey's medium was substituted for the CLED medium, and was found to be satisfactory. The change to MacConkey's medium, however, caused an alteration in the relation between the viable count and the number of colonies per spoon.

In Mackey and Sandys' outfit the spike of the spoon was impaled upon a pad of moist cotton wool to immobilise the spoon in transit, and to absorb any urine which drained from it. However the cotton wool was troublesome in practice. When preparing the spoons it was difficult to gauge the right amount to use, and flecks of cotton wool tended to rise up and adhere to the surface of the medium. These difficulties have been overcome by the use of a polyurethane foam wad 25 mm in diameter and 7½ mm thick in place of the cotton wool. These were supplied by Cindy Foam Ltd. Instead of the 2 ml of water

used to moisten the cotton wool, only 0.5 ml was required for the foam wad, which was less absorbent.

Organisms and media

For the laboratory experiments organisms isolated from specimens of urine submitted to the routine medical laboratory at the University of Aberdeen were used. Strains of Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa and swarming strains of Proteus mirabilis were investigated. The identity of the organisms was established by the tests listed in Table 4.

The CLED medium was prepared according to the original method (Sandys, 1960). All the other media used was prepared as detailed in Section 1.

Preparation of the spoons

Preparation of the spoons was laborious, and was carried out entirely by the author. The foam wad was put into the screw-topped container, and pushed to the bottom, and 0.5 ml of water added with an automatic pipette. The top was then screwed on to the container which was autoclaved at 121°C for 15 minutes. At the same time the spoons and a rack upon which the spoons were to be placed for filling were also autoclaved.

A suitable quantity of MacConkey's medium was melted and cooled to 56°C. In order to get spoons filled so that the surface had the proper convexity, it was important that the medium was not too hot, or it contracted and evaporated as it set. The spoons were then set out on the rack with flamed forceps, and filled individually from a 10 ml pipette. Various attempts at automation were made, but without success.

When the agar had set (during which time the spoons were open to the atmosphere) each spoon was inserted into a bottle with flamed forceps.

The spoons were then incubated for 48 hr at 37°C, and afterwards stored at room temperature for three weeks before issue. No attempt was made to examine the spoons until the outfits were being prepared for issue, and no record was kept of the degree of contamination.

Testing the spoons

The viable count of an overnight nutrient broth culture of the organism under test was determined by Miles and Misra's method (Miles, Misra and Irwin, 1938) (see page 99). Doubling, fourfold and tenfold dilutions of the culture were made with 0.85 N NaCl. Several spoons were then dipped into each dilution, returned to their containers, incubated overnight, and the number of colonies counted the next morning. The number of colonies per spoon (the spoon count) was then related to the viable count of the diluted specimen.

In one experiment the effect of diluting the cultures with distilled water or nutrient broth was investigated. In another the pH of the culture, diluted with nutrient broth, was adjusted to values of 5, 6, 8 and 9 by the addition of 0.1 N NaOH or 0.1 N HCl, the pH being measured by a Beckman pH meter. Spoons were dipped into the diluted culture and the effect of pH on the relation of the spoon count to the viable count investigated.

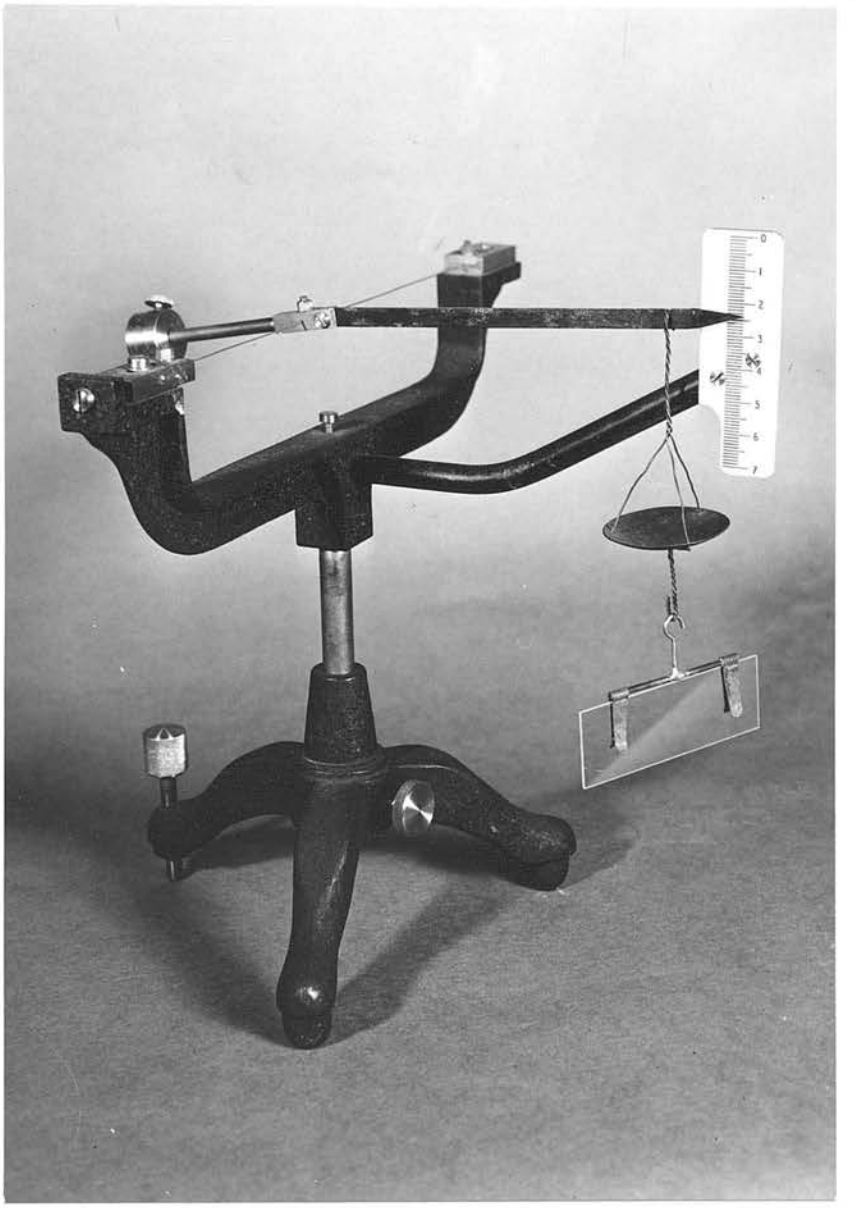
Surface tension

The surface tension of various cultures, and of de-ionised water, ether, chloroform and glycerine was measured by Wilhelmy's method by which the surface tension acting on a glass slide suspended in a balance is estimated by the weight needed to counteract it (Figure 7).

All glassware was carefully washed and then immersed for at least 12 hours in suitably diluted 'Decon 75' (a commercial cleansing and decontaminating agent manufactured by Medical Pharmaceutical Developments Ltd) for degreasing.

FIGURE 7

Balance for measuring surface tension



The surface tension was calculated from the following formula:

$$\text{Surface Tension} = \frac{Mg}{2l} \text{ dynes per cm}$$

where M = Mass (grams) added to the balance to equal the effect of the surface tension,

l = length (cm) of the glass slide,

g = force of gravity (981 cm per sec^2)

Care was taken to dry the glass slide in air between each measurement, and a new slide was used whenever a different liquid was being tested. As the surface tension varies significantly with temperature care was taken to standardise the temperature both of the room and the liquid under test to between 20°C and 22.5°C.

Ultrasonic experiments

Ultrasonic waves were used to disrupt clumps of cocci (Staphylococcus aureus)in an overnight broth culture, and the relation between the spoon count and the viable count (by Miles and Misra's technique) was investigated.

Preliminary experiments were carried out to determine the optimum length of ultrasonic treatment, and an experiment with a strain of E. coli was included as a control. An MSE 100 watt ultrasonicator was used. Five ml of a six hour broth culture were placed in the special container, and 0.1 ml removed immediately and used to estimate the untreated viable count. After each period of ultrasonic treatment a further 0.1 ml was removed for a further estimate of the viable count. A minimum amplitude of 8 microns peak to peak vibration was maintained throughout the experiment, and to prevent heating of the culture it was cooled in an iced-water jacket.

After the preliminary experiments a strain of Staphylococcus aureus was treated in the ultrasonicator for 4 minutes, and the relation between

the spoon count and the viable count of the treated and untreated specimens was investigated by diluting the samples with 0.85 N NaCl and dipping spoons into different dilutions as detailed above.

Stream-inoculation

A disadvantage of the dip-inoculum method as proposed by Mackey and Sandys is that two containers have to be used, one in which to collect the specimen of urine, and the other to hold the spoon. Inoculation of the spoon by passing it through the uninterrupted stream of urine would eliminate the need for a second container, and it would also dispose of the considerable practical and aesthetic difficulties of obtaining proper mid-stream specimens of urine from some patients, and, not least, it is more likely that the specimen sampled would, in fact, be from the middle portion of the stream.

The considerable differences in the force and rate of flow between the sexes, between patients of different ages, and between those with severe cystitis and urethritis and those with a healthy lower urinary tract impose a further set of variables which have to be taken into account. To compare the stream method with the dip-inoculum method, and to estimate the effect of the variable factors mentioned above, a simple model bladder was set up. This consisted of a flask containing a suspension of organisms which was voided by a siphon through an orifice 4 mm in diameter. By raising or lowering the flask the rate of delivery of the urine could be altered. Two extremes of rate of delivery were investigated a rate of 3 ml per second (a trickle) to simulate a severe cystitis, and a rate of 35 ml per second to simulate a healthy male type of voiding.

The viable count of the suspension in the bladder was estimated by Miles and Misra's technique (see page 99). The spoons were passed briefly through the stream, and after incubation, the spoon count was related to the viable count in the bladder.

Clinical trials

After establishing the relation between the viable count and the spoon count in the laboratory trials it was desired to extend the trial to clinical material. As a preliminary guide 196 urines received at the bacteriology laboratory from hospital patients or outpatient clinics were examined in parallel by the dip-inoculum method, and by the standard loop method of McGeachie and Kennedy (1963) which is the routine practice in this laboratory. In the normal course of events urines examined in the laboratory by this semi-quantitative method are reported as being in one of the following categories: "No growth", "Less that 10^4 organisms per ml", "Between 10^4 and 10^5 organisms per ml" or "Over 10^5 organisms per ml". The viable counts estimated by the spoon method (not the spoon count) were similarly grouped, except that for the purposes of this trial the "No growth" group was included with the group labelled "Less than 10^4 organisms per ml".

Following this, and with the co-operation of the antenatal clinic of the Aberdeen Maternity Hospital 297 Mid-stream specimens of urine which were collected in the usual way were 'dip-inoculated' by the nursing staff, and the inoculated spoon was sent to the laboratory along with the specimen of urine. A sheet of instructions was prepared (see Appendix 2) and issued to the nurses involved, and the co-operation achieved was excellent.

In a later series from the same clinic the stream inoculum method was tested. Patients were instructed by the nursing staff to hold the spoon briefly in the stream of urine and then replace it carefully in its container. A mid-stream specimen was also obtained, and both specimens were dispatched to the laboratory where they were examined and reported as before.

Finally the stream-inoculum system was used in a clinical trial of the treatment of urinary tract infection in general practice. Fifteen general practitioners in six practices took part and there were 530 patients. The patients were instructed by the practitioner on the method of inoculating the spoon, but usually carried out the procedure privately. Three weeks after, and again in most cases seven weeks after, the initial specimen was received follow-up specimens were posted to the patients in their homes for inoculation and returned to the laboratory. The only difficulty noted apart from the fact that a proportion failed to return the specimen, was that 5 per cent. of specimens were returned to the laboratory with the container filled with urine.

RESULTS

Relation between spoon count and viable count for E. coli

The relation between the colony count on the dip-inoculum spoons (spoon count) and the viable count of a strain of E. coli estimated by Miles and Misra's method is shown in Figure 8. A total of 310 observations were made in all. Of these 45 spoons had no growth, all from dilutions containing less than 1×10^4 organisms per ml, and 72 spoons had a confluent growth, all from dilutions containing more than 2.5×10^5 organisms per ml. The remaining observations are plotted on the graph.

The relation is that of a straight line. There are wide variations, but for values of the viable count over 1×10^4, where there was a ten-fold difference in the viable count there was no overlap in the number of colonies per spoon. The 95 per cent. confidence limits for the slope were obtained by the method of weighted least squares on the assumption that the variance of the spoon counts was proportional to the viable count (or that the standard deviation was proportional to the viable count). The line of best fit through the origin had an estimated slope of 0.9147×10^{-3}. Ninety five per cent. confidence limits of this line are 0.9949 and 0.8345, i.e. $\pm$ 0.0802. In practical terms 9 colonies per spoon corresponds to a viable count of 10^4, and 91 colonies to a count of 10^5 bacilli per ml.

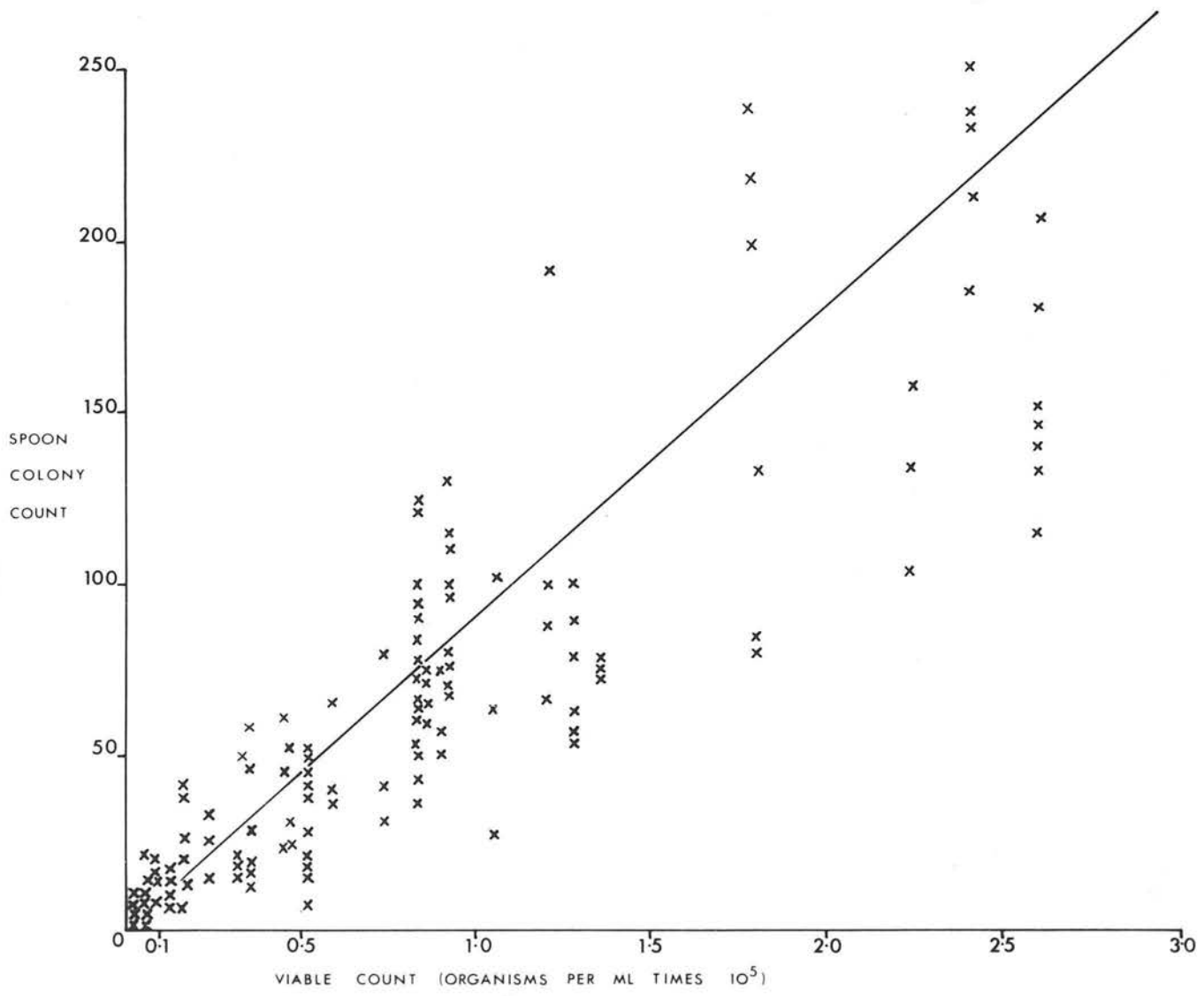
Surface tension

At an early stage in the investigation it became apparent that there were many more colonies per spoon for a given viable count of Staph. aureus than for the same count of E. coli. A possible explanation for this could be that the surface tension of a culture may be altered by the products of bacterial growth, so that more or less liquid would be retained on the spoon following its

FIGURE 8

Relation between the viable count and the spoon colony count of strains of E. coli

Where the points on the graph are closely clustered, and especially near the origin of the graph, some points have been ommitted for the sake of clarity.


250
200
150
100
50
0
SPOON
COLONY
COUNT
0·1
0·5
1·0
1·5
2·0
2·5
3·0
VIABLE COUNT (ORGANISMS PER ML TIMES 10^5)

withdrawal from the liquid. Even although in the laboratory experiments cultures were diluted it needs only a small number of molecules of a suitable substance to alter the surface tension significantly.

The results of measuring the surface tension of the control liquids (de-ionised water, ether, chloroform and glycerine) were uniformly, and quite consistently high. In spite of considerable effort and consultation with a physicist the source of the error could not be found. However since absolute values were not important, it was decided to continue the experiment to establish whether or not there was a significant difference between cultures of Staph. aureus and E. coli. The results of the experiment are recorded in Table 39, no significant difference between the surface tensions of the cultures could be observed. Moreover as the culture was diluted its surface tension approximated to that of water so that the surface tension of a 1 in 1000 dilution of either E. coli or Staph. aureus was indistinguishable from water. Such a dilution of an overnight culture will contain 10^4 or 10^5 organisms per ml, and dilutions of this and greater magnitudes were used for testing the dip-inoculum spoon.

Clearly differences in surface tension could not account for the different relation between the viable and spoon counts of E. coli or Staph. aureus.

Disruption of clumps of Staph. aureus

The disruption of clumps of staphylococci as they passed over the spoon was another possible mechanism to account for the greater number of colonies per spoon for a given viable count of a culture of Staph. aureus than for the same viable count of a culture of E. coli.

To investigate this possibility cultures of Staph. aureus were subjected to ultrasonic waves to disrupt the clumps. Preliminary experiments, however,

TABLE 39

Surface tension of cultures of E. coli and Staph. aureus in nutrient broth

Material under test	Temperature (°C) of the material	Temperature (°C) of the room	M = mass (gm) reqd. to overcome surface tension	Surface tension ($ST = \frac{Mg}{21}$** dynes per cm)
*Controls:				
Sterile de-ionised water	-	-	1.33	84.7
Ether ($C_4H_{10}O$)	-	-	0.37	23.6
Chloroform ($CHCl_2$)	-	-	0.55	35.1
Glycerine ($C_3H_6O_3$)	-	-	1.25	77.5
E. coli				
Overnight culture (1)	22.5	20.5	0.91	56.5
Overnight culture (2)	22.0	21.5	1.02	63.4
1 in 10 dilution of above	21.0	22.5	1.12	69.5
1 in 100 dilution of above	21.0	22.0	1.27	78.5
1 in 1000 dilution of above	21.0	22.0	1.33	84.7
Staph. aureus				
Overnight culture (1)	22.0	21.5	1.02	63.4
Overnight culture (2)	21.0	22.0	1.02	63.4
1 in 10 dilution of above	21.5	21.5	1.19	74.0
1 in 100 dilution of above	21.0	21.0	1.33	84.7
1 in 1000 dilution of above	20.0	21.0	1.34	85.5

* Mean results for five estimations for each liquid. The room temperature and the temperature of the liquid was in every case between 20°C and 22°C.

** g = 981 cm per sec^2: l = 7.7 cm.

had to be carried out to determine the optimum length of ultrasonic treatment, and the results of these are shown in Figure 9. The single strain of E. coli used as a control showed an uninterrupted decline in numbers from 2.1×10^8 to 4.5×10^5 in four minutes. The strains of Staph. aureus showed an initial rise in the viable count followed by a decline. The extent of the increase in the viable count was about two-fold, and a decline in the count had begun after four minutes or earlier. In the initial few minutes the breaking up of the clusters of cocci, which by Miles and Misra's technique of viable counting would be indistinguishable from a single organism, resulted in an increase in the count, until the lethal effect of the ultransonic waves overtook the declumping effect causing a decrease in numbers.

A period of four minutes of ultrasonic treatment was thought to be the most suitable, for by this time it was considered that most of the clumps would have been broken up, but the damage to the individual cocci would not have reached severe proportions.

Relation between spoon count and viable count for Staph. aureus

The relation between the viable count and the spoon count of cultures of Staph. aureus some of which were treated with ultrasonic waves for four minutes, and some of which were untreated is shown in Figure 10. Three hundred and ninety eight observations were made in all, including 61 with confluent growth and 83 with no growth which are not shown on the graph. Clearly from the figure, for corresponding values of the viable count the number of colonies per spoon tends, for values above 2.5×10^4, to be higher for untreated cultures, and the linear regression for these values will have a greater slope than that for the treated culture. Indeed there is some

FIGURE 9

Effect of ultra-sonic waves on strains of Staph. aureus and E. coli

The viable count of the strain of E. coli shows a rapid and uninterrupted decline during exposure to ultra-sonic waves.

Strains of Staph. aureus show an initial increase in the viable count corresponding to 'declumping', followed by a gradual decline in numbers as lysis overtakes 'declumping'.

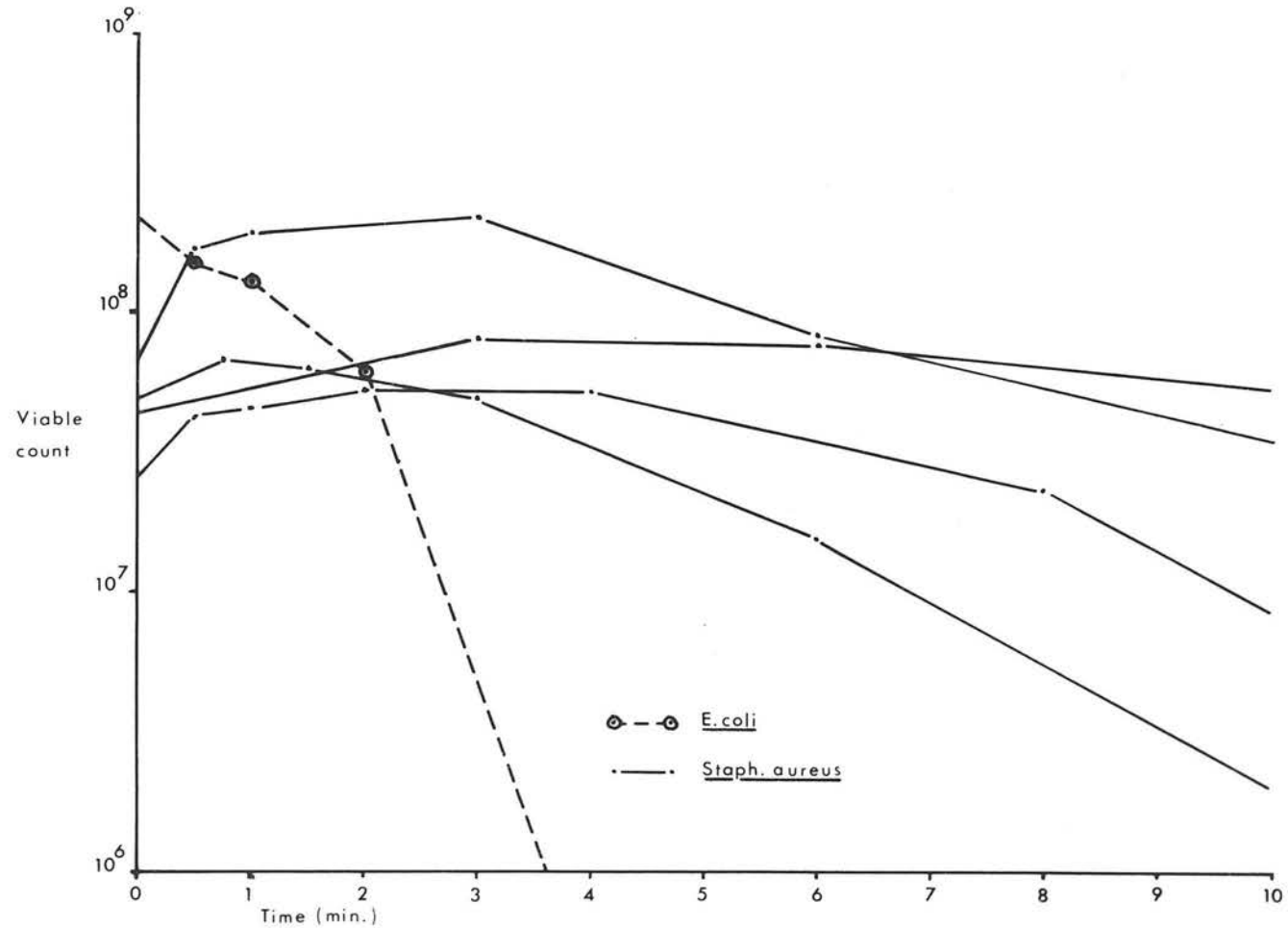
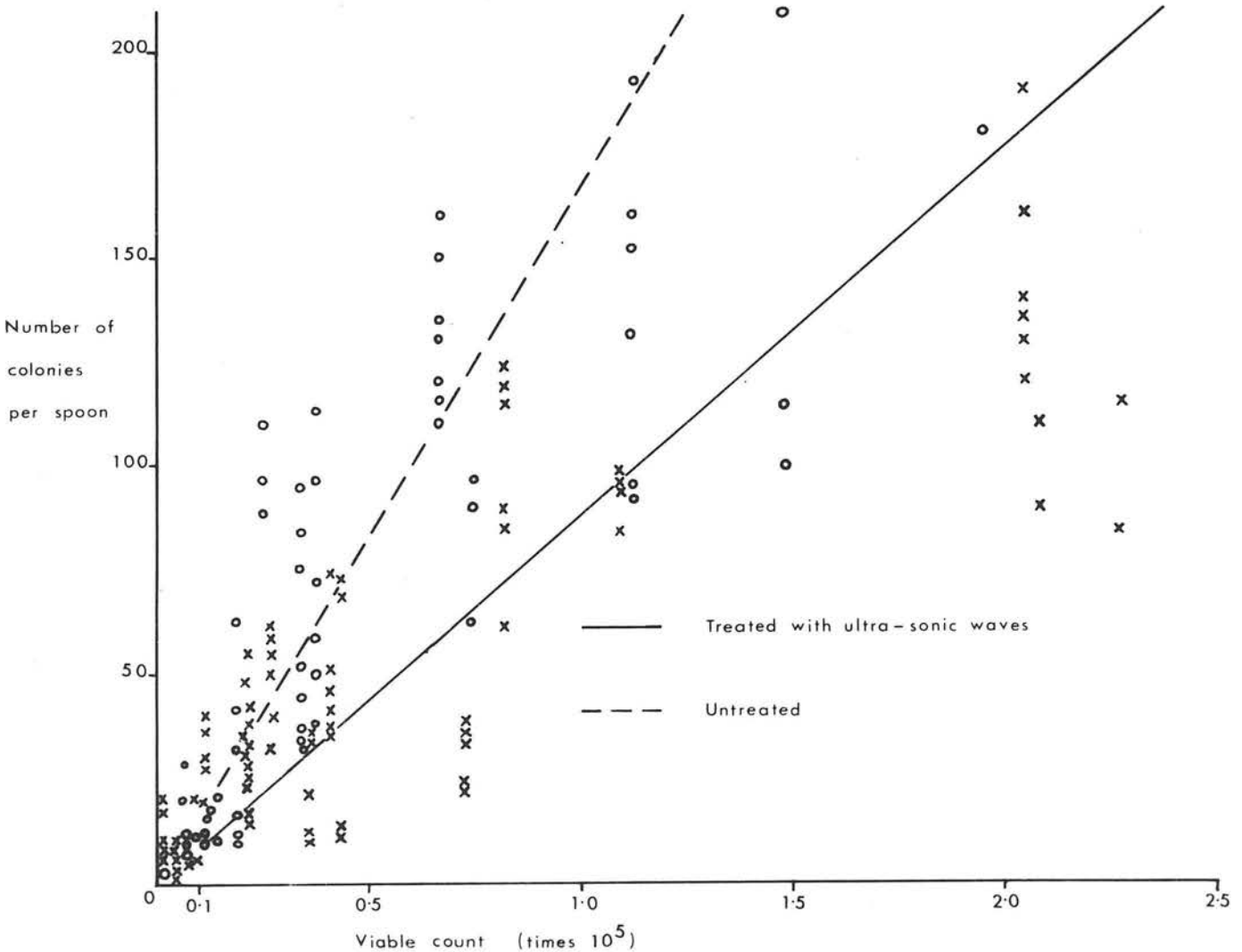

10^9
10^8
10^7
10^6
Viable count
E.coli
Staph. aureus
0
1
2
3
4
5
6
7
8
9
10
Time (min.)

FIGURE 10

Relation between the viable count and the spoon colony count of strains of Staph. aureus before and after treatment with ultra-sonic waves

Where the points on the graph are closely clustered, and especially near the origin, some points have been ommitted for the sake of clarity.


200
150
100
50
0
Number of colonies per spoon
0·1
0·5
1·0
1·5
2·0
2·5
Viable count (times 10^5)
Treated with ultra-sonic waves
Untreated

evidence from the data that a quadratic curve would give a better fit to each set of data, and such a curve for the untreated culture would also clearly lie above that for the treated culture. Linear or quadratic curves could be fitted by the method of weighted least squares to allow for increasing variability of the higher counts, but the labour is considerable, and a statistician has expressed the view that the differences are clear-cut for these data, and that the labour would not be justified merely to confirm that there is a difference.

Relation between spoon count and viable count for different species of bacteria

The relation between the spoon count and the viable count for different species of bacteria under a variety of conditions is shown in Table 40 and it includes, for comparison, data compiled from Figures 8 and 10. The table has been prepared by plotting each set of results and fitting the best straight line through the origin to each set of results, and then reading from the graph the number of colonies per spoon which corresponds to 10^5 bacteria per ml.

It is clear that the relation varies considerably depending on the organism involved and on the conditions of the test. Under similar conditions for cultures of E. coli, Str. faecalis, Proteus and Staph. aureus (after ultrasonic treatment) the mean number of colonies per spoon for a viable count of 1×10^5 lay between 80 and 100 colonies, but untreated cultures of Staph. aureus gave many more colonies per spoon (165), and cultures of Ps. aeruginosa gave many fewer (38).

Diluting a culture of E. coli with nutrient broth or distilled water instead of 0.85 N NaCl did not significantly alter the ratio between the

TABLE 40

Relation between the spoon colony counts in different circumstances and the viable count

Organism	Method of inoculation	Number of observations	Conditions*	Spoon count when viable count was 1×10^5
E. coli	Dip-inoculation	310	Standard	91
		154	Spoon filled with CLED medium	43
		31	Culture diluted with distilled water	91
		52	Culture diluted with nutrient broth at pH 7	91
		45	pH adjusted to 5	144
		26	pH adjusted to 6	115
		26	pH adjusted to 8	90
		36	pH adjusted to 9	100
Staph. aureus	Dip-inoculation	213	Standard	165
		185	Culture treated with ultrasonic waves	88
		105	Spoon filled with CLED medium	128
Str. faecalis	Dip-inoculation	145	Standard	80
Proteus	Dip-inoculation	61	Standard	100
Ps. aeruginosa	Dip-inoculation	44	Standard	38
E. coli	Stream-inoculation	50	Fast stream	42
		59	Slow stream	61

* The standard conditions under which the tests were conducted were:
1. The overnight culture was diluted with 0.85 N HaCl. 2. The pH was 7.
3. The spoon was filled with MacConkey's medium.
Any departure from these conditions, or any additional procedure is noted.

number of colonies per spoon and the viable count, but the use of CLED medium in the spoon instead of MacConkey's medium caused a reduction in the number of colonies per spoon for the same viable count with cultures of E. coli and Staph. aureus. Alteration in the pH had a profound effect, reducing it to pH 6 and then to pH 5, and increasing it to pH 9 all increased the number of colonies per spoon for a given viable count, while increasing the pH to 8 did not alter the count significantly.

The effect of stream-inoculation was to reduce the number of colonies per spoon for a given viable count compared with dip-inoculation. When the stream was slow this reduction was about 33 per cent. (from 91 colonies to 61 colonies per spoon for the viable count of 1×10^5), but with a fast stream the reduction was marked so that only 42 colonies represented a viable count of 1×10^5 bacteria per ml.

Criteria used when reporting viable counts by the spoon method

The dip-inoculum or stream-inoculum spoon method of carrying out a viable count can be classed only as a 'semi-quantitative' technique. When recording clinical specimens either to a clinician or for the purposes of this investigation, each result was therefore, allocated to a category, and reported as being within that category. The categories were (as reported above) "Less than 10^4 organimsm per ml", including those specimens with no growth, "Between 10^4 and 10^5 bacteria per ml", and "Over 10^5 bacteria per ml".

The criteria used to assign a spoon specimen into one of the three categories are set out in Table 41. In fact there were no isolates of Ps. aeruginosa from the clinical material, and very few isolates of

Staph. aureus (less than 1% of isolates), so for practical purposes only the last two rows of figures in the table were important. Even so the counting of colonies was only rarely required. The very great majority of spoons had either less than 5 colonies, or a confluent or semi-confluent growth.

Although the area of the agar covering the spoon is small (about 320mm^2), the number of colonies that can be counted upon it are large. When the colonies are few each one is large, but they become smaller as their number increases until they become semi-confluent (confluent on parts but not all of the spoon, the lower portion being affected first usually) and finally confluent.

TABLE 41

Criteria used when reporting spoon specimens

Organism	Method of inoculation	Number of colonies per spoon equivalent to a viable count of	
		10^4 organisms per ml	10^5 organisms per ml
Staph. aureus	Dip	16	160
	stream	11	110
Ps. aeruginosa	Dip	4	40
	stream	3	30
All other organisms	Dip	8	80
	stream	5	50

Colonies of E. coli remain distinct until their number reaches 250 or so, but more than 500 discrete colonies of Str. faecalis or Staph. aureus may be counted.

The distribution of colonies upon the spoon is not always uniform, the lower half often having more colonies than the upper half, but this is by no means a rule. Accurate counting of more than 50 or 60 colonies per spoon requires the use of a hand lens, If it were being done on a very large scale a special viewer incorporating a lens or low power plate microscope would save trouble and increase accuracy.

Proportion of clinical specimens in each 'growth' category

The proportion of clinical specimens in each 'growth' category is recorded in Table 42 for each series of specimens. Allocation to a category for this table has been determined by the result obtained by the routine laboratory using a standard loop semi-quantitative method. The proportion of specimens that were negative was lowest (34 per cent.) in the group of selected patients

TABLE 42

Proportion of clinical specimens in each 'growth' category

Number and source of the specimens	Number (and %) of specimens in which the viable count given by the standard loop method was		
	over 10^5	between 10^4 and 10^5	under 10^4
196 midstream urines from hospital patients	53(27)	17(9)	126(64)
297 midstream urines from patients attending an antenatal clinic	54(18)	16(5)	227(77)
70 specimens from selected patients attending an antenatal clinic	41(59)	5(7)	24(34)

from the antenatal clinic, and highest (77 per cent.) from larger group of antenatal patients only a few of whom were selected.

Use of dip- and stream-inoculum spoons with clinical specimens

The results of the comparison of the dip-inoculum and stream-inoculum spoons with the standard loop method of performing a semi-quantitative viable count used by the routine bacteriology laboratory are recorded in Table 43. In approximately 90 per cent. of all specimens the spoon count and 'routine' viable count correspond.

In approximately 10 per cent. of cases a difference was recorded between the two methods. A proportion of these cases is due to specimens with viable counts close to either 1×10^5 or 1×10^4 which have been assigned to different categories by the two methods. There is however a clear tendancy for the spoons to give a higher estimate of the viable count than the standard loop method, but it is not possible to establish whether the spoon methods were over-estimating or the standard loop method under-estimating.

In the few cases where there was a difference of more than 10 fold further explanation is required. Firstly, although the categories differ by 100 fold, specimens in the different categories may differ by only a little over 10 fold. In the two cases where the spoons gave low results, neither spoon showed any growth, and it is possible that they were not inoculated. In the $1\frac{1}{2}$ - 2 per cent. of specimens where the spoon indicated a viable count more than ten times that indicated by the standard loop the explanation may lie in a combination of the expected statistical errors associated with both semi-quantitative methods, or to technical errors in carrying out either the spoon count or the standard loop count, or finally in perhaps one or two cases to the lethal effect of an acid urine or of antibiotics in the urine killing the bacteria between the time of inoculating the spoon and dealing with the specimen in the routine laboratory.

TABLE 43

A comparison of the viable count estimated by dip- or stream-inoculation with that estimated by a standard loop method

Number, source and method of inoculation of the specimens	Number and proportion (%) of specimens in which the viable count given by the spoon method was				
	the same as that given by the standard loop	in a different category from that given by the standard loop and was			
		100 times higher	10 times higher	10 times lower	100 times lower
196 midstream urines from hospital patients dip-inoculated after normal laboratory procedures were completed	170 (87)	5 (2.5)	17 (8.7)	4 (2)	0
297 midstream specimens from patients attending an antenatal clinic. Spoon dip-inoculated in the clinic and spoon and urine dispatched to the laboratory	270 (91)	5 (1.7)	16 (5.4)	4 (1.3)	2* (0.6)
70 specimens from antenatal clinic patients. Spoon stream-inoculated by patient. Spoon and midstream urine sent to laboratory.	65 (93)	1 (1.4)	2 (2.8)	2 (2.8)	0

* These specimens may not have been inoculated.

Use of stream inoculum spoons in general practice

In general medical practice where the treatment of uncomplicated urinary tract infection without bacteriological control is accepted as normal (Eykn and Phillips, 1969) any proposed system of urine culture must be reasonably accurate, administratively easy for the practitioner, and acceptable to the patients if it is to gain widespread approval. The stream inoculum system fulfils these criteria. In the clinical trial 530 first specimens were received. Second specimens were requested from 522 patients and in all but 21 it was provided (96 per cent.). Only 303 third specimens were requested, and 232 patients obliged (77 per cent.). There was no obligation to inoculate and return the specimen, and no visit from the general practitioner was made or threatened, and so it is considered that the system was indeed acceptable to the patient.

In all 1287 specimens were received (fourth and fifth specimens were obtained from a few patients) and 5.3 per cent. (67) of these were incorrectly inoculated, having free urine in the bottle. This represented 2 per cent. of first specimens which were taken after instructions from the general practitioner, and must represent the irreducible minimum. Except for those who had previously supplied a bottle with urine in it further instructions were not issued with the second and third specimens, because it was not considered necessary at first, and then because of the difficulty in wording the instructions so as not to cause offence or disgust. The proportion of specimens incorrectly inoculated increased to over 5 per cent. of the second specimens and nearly 10 per cent. of the third specimens.

Questionaire to general practitioners

Before taking part in the trial which involved the use of the stream

TABLE 44

The results of a questionaire on the use of the stream-inoculum transport outfit by 11 general practitioners

Question	Yes	No difference	No
Is the spoon outfit better than the traditional method of urine transport?	3	8	0
Is the spoon outfit better than the boric acid method of urine transport?	1	7	3
Do you consider that pus cell counts are a useful guide in most circumstances?	10	0	1

inoculum spoons the practitioners involved sent 'natural urines' for bacteriological analysis to the City hospital laboratory in Aberdeen. These specimens contained no preservative nor were they refrigerated. During the period of the trial the use of boric acid to preserve specimens of urine was introduced by that laboratory, and so following the trial these practitioners used the boric acid method, and are therefore well qualified to comment on the relative merits of the three methods.

The results of the questionaire are shown in Table 44. Surprisingly the practitioners did not consider that there was much difference between the various methods tried although there was a slight shift of opinion in favour of the stream-inoculum spoon against the use of 'natural urines', and in favour of the use of boric acid preservative at the expense of the spoon method.

Almost unanimously they considered that a pus cell count was useful,

and this is of course possible with the boric acid method, but not with the spoon method. Moreover the boric acid method allows albumin and glucose to be estimated by the laboratory, and some of those practitioners who had become used to getting this service when 'natural urines' were sent to the laboratory commented upon its non-availability with the spoon method and were grateful to take advantage of it again after the trial was over.

DISCUSSION

Any rapid cheap method of carrying out a viable count sacrifices a degree of accuracy, and the dip-and stream-inoculum methods are no exception. The critical range of bacterial counts in urine is between 10^4 and 10^5 bacteria per ml, counts of less than 10^4 being usually due to contamination, and those of over 10^5 being usually due to infection (Kass, 1956). Urquhart and Gould (1965) considered that, "a degree of accuracy of the order of 10 fold, i.e. one log, seems adequate to distinguish between counts in the critical urinary range of 10^3 to 10^6 organisms per ml". The results indicate that the dip-and stream-inoculum methods can achieve this degree of accuracy. Urquhart and Gould continue, "Where the counts fall into the doubtful range of 10^4 - 10^5 bacteria per ml, assessment of a series of samples is always indicated". Where the object of such an assessment is to cure patients of urinary infection (if present), rather than to select subjects with proven infection for a clinical trial, the clinician must bear in mind that bacterial excretion may be intermittent and slight (Stamey et al., 1965; Ambrose and Hill, 1965).

Spoon counts and viable counts for various organisms

The results show that the relation between the spoon count and the viable count for the different organisms were closely similar except for cultures of Staph. aureus and Ps. aeruginosa, with about 80 colonies per spoon representing 10^5 bacteria per ml. Cultures of Staph. aureus, however, gave many more colonies per spoon for a given viable count than did other organisms, and this contradicts the findings of Mackey and Sandys (1965) who noted that Gram-positive cocci (unspecified) gave fewer colonies per spoon than did Gram-negative bacilli. The explanation for this apparent contradiction lies firstly in the results of

the treatment of the cultures of Staph. aureus with ultrasonic waves, which showed that 'de-clumped' cultures gave results similar to cultures of E. coli, Proteus and Strep. faecalis, and also in the methods employed to measure the viable count in this survey and in Mackey's investigation. In the latter study the viable count was performed by spreading 0.1 ml of dilutions of the culture over the whole of an agar plate with a loop. This technique probably achieves quite a considerable degree of de-clumping. With the Miles and Misra technique, employed in the work for this thesis, there would be little or no de-clumping. An intermediate degree of de-clumping probably occurs during dip-inoculation, the clumps of cocci breaking up as they pass over the surface of the spoon. It appears therefore that the anomalies in the relation between the spoon count and the viable count of staphylococci are related to clumping which is a characteristic feature of this organism. No such anomaly was noted with cultures of Strep. faecalis.

There were fewer colonies per spoon for a given viable count of a culture of Ps. aeruginosa than for any other organism. No explanation can be offered for this, but it may be noted that only one culture was examined, and only a few spoons (35) were counted. Clearly further cultures of this organism need to be investigated.

Effect of surface tension

The results of the experiments in which the surface tension of cultures of E. coli and Staph. aureus was measured indicated that there were no significant differences in the surface tensions of cultures of these two organisms, and that alterations in the relation between the spoon count and the viable count could not be due to this factor.

Effect of surface charge

The marked changes in the relation between the spoon count and the viable count induced by altering the pH of the culture and by substituting MacConkey medium for CLED medium in the spoon suggests that the charge on the surface of the bacteria and the charge on the surface of the medium are important in causing bacteria to adhere to the medium as the spoon is withdrawn from the culture. Information about the surface charge of bacteria is meagre, but Harden and Harris (1953) measured the isoelectric point of a number of strains of bacteria, and they reviewed and correlated the findings of others. From these results it would appear that closely related organisms can differ significantly in their isoelectric point, and it may be that different strains of the same species may have different isoelectric points. If there are significant differences in the charge on the surface of different strains of bacteria this factor may contribute to the considerable variation in the spoon count (for a given viable count) which was noted for different strains of the same species.

Accuracy of the spoon count

Most of the dip- and stream-inoculum spoons were, after incubation, either obviously negative, with few or no colonies upon them or were obviously positive with a semi-confluent or confluent growth. In only a small proportion was there any doubt about the category in which the specimen should be placed, and only these spoons required careful counting of the colonies.

Two factors must be recognised when considering the number of cases in which a difference between the standard loop method and the spoon method of viable counting was recorded. (1) Two semi-quantitative techniques were compared with each other, and not against an absolute standard. (2) The method

of reporting growth in categories ("Less than 10^4", "Between 10^4 and 10^5, and "Over 10^5 organisms per ml") inevitably results in some specimens that are borderline between two categories being reported in different categories although the viable counts estimated by each method do not differ significantly. Under these circumstances a correlation between the two methods of 87 to 93 per cent. was satisfactory.

Comparison of dip-inoculum and stream-inoculum methods

The stream-inoculum system gave fewer colonies for a given viable count than did the dip-inoculum method, and unfortunately the number of colonies varied with the rate of flow of urine. Furthermore, since the difference between the number of colonies per spoon representing 10^4 and 10^5 organisms per ml was less (45 colonies compared with 72 colonies with the dip-inoculum method, see Table 41) and as the scatter did not seem to be altered (although this was not measured), the inaccuracy of the stream-inoculum method was theoretically greater than the inaccuracy of the dip-inoculum spoon. However in the clinical trial in which 70 urines from antenatal patients were examined, and in which 64 per cent. of the specimens recorded a growth of more than 10^4 bacteria per ml, the stream-inoculum system was as accurate as the dip-inoculum system had been in a previous trial.

Commercially produced spoons

The preparation of the spoons in the laboratory was time consuming, tedious, and probably uneconomic, although no serious attempt was made to estimate the cost of each outfit. Recently some of the pharmaceutical companies have realised the commercial possibilities in the system, and are launching large publicity campaigns to encourage general practitioners and

hospital doctors to use them. The outfits produced by the two major companies in this field are competitively priced at 7½ p (1/6d) and both incorporate two media - the CLED medium and nutrient agar to facilitate identification and counting of the colonies. However, although the difficulties which the author experienced with the CLED medium may not be entirely relevant with a different outfit, it must still be questioned whether the advantages claimed for the new medium (Mackey and Sandys, 1965), justify the change from MacConkey's medium with which all are familiar. Furthermore the use of two media to assist, presumably, in the identification and accurate counting of the colonies, has only dubious advantages. Since any organism present in confluent or semi-confluent growth will have to be plated out to ensure that there is no mixture if for no other reason, accurate identification of the colonies on the spoon is not essential. Also any inaccuracies due to the slightly inhibitory nature of MacConkey's medium will result in only relatively small errors. A cheaper outfit, or perhaps a single thicker layer of agar on the spoon might be better value. Some of the commerical outfits are based on a microscope slide coated with agar on part of both sides. The surface area of the agar is about 12 cm^2 in all, and requires a considerable amount of urine for immersion, more than can be obtained from a child or an adult with stranguary, in a mid-stream specimen. Since it is really the density of the colonies on the slide, and not their total number which is determined by the viable count and is the cause of the difficulties associated with a confluent growth, an outfit with a large area of agar has no advantage. In fact for the bacteriologist who has to count the colonies, it has a distinct disadvantage.

Clinical advantages and disadvantages of the dip-inoculum and stream-inoculum outfits

From the clinician's point-of-view the dip-inoculum method has a place in modern medicine only when difficulties arise between getting the specimen from the patient and inoculating it on to the culture media in the laboratory. It eliminates the inaccuracies which arise when a specimen is delayed or un-refridgerated in transit. Its use does not make the collection of the specimen any easier, and indeed the requirement for two containers, only one of which is usually supplied by the laboratory, has in the author's experience resulted in the use either of the container which should be reserved for the spoon (the urine being poured from the container before transport of the outfit to the laboratory) or of other even less desirable containers, which may be un-sterile, messy to use, or so large that the whole of the specimen is usually collected. The use of a urine preservative such as boric acid (Porter and Brodie, 1969) can achieve the same result more cheaply and allow the measurement of albumin and glucose, and the examination of the urine for pus cells, provided sufficient urine can be passed to fill the container to the brim.

The stream-inoculum method simplifies the collection of the specimen. It is clean to use and easy to sample the mid-portion of even a short stream of urine without discomfort to the patient, and furthermore, a second container is not required. Its disadvantages are that some degree of re-education of nursing staff and patients is required for its proper use, and secondly that it may be slightly less accurate than the dip-inoculum spoon.

In general practice, where the treatment of uncomplicated urinary tract infection without bacteriological control is accepted as normal and justified (Eykn and Phillips, 1969), any proposed system of regular urine culture must be reasonably accurate,

administratively easy, acceptable to the patient and able to be used under difficult circumstances if it is to gain widespread approval. Only the stream-inoculum method satisfies these criteria fully. Although unfavourably commented upon by the practitioners involved, the lack of a pus cell count is not a great disadvantage. The presence of pus cells does not indicate that a urinary infection is present, nor does their absence preclude it (Brumfitt, 1964; Smallpiece, 1966; Mond et al., 1970).

Bacteriological advantages and disadvantages

The bacteriologist will find that while the commercial dip- or stream-inoculum outfit is probably cheaper than the cost of two agar plates and the cost of the container used to transport the specimen to the laboratory, and probably much cheaper when the cost of the time spent seeding plates with urine is counted, nevertheless if the majority of the specimens is positive, or if a large proportion come from sources which previously did not use the laboratory, he will find that his work load is increased.

When the number of positive specimens is large compared with the total, the frequent necessity to sub-culture from the confluent growth in order to get single colonies for identification and sensitivity testing will erode some of the advantage of being able to discard negative specimens without carrying out any technical procedures upon them. Difficulties also arise if two or more organisms are present when the growth is confluent or semi-confluent. The presence of a mixture may be missed, and when it is discovered it is usually difficult and often impossible to determine which was the dominant organism.

SUMMARY AND CONCLUSIONS

1. In this section the use of the dip-inoculum spoon has been evaluated. The relations between the spoon count and the viable count of strains of E. coli, Proteus, and Strep. faecalis were similar, but cultures of Staph. aureus showed more colonies per spoon for a given viable count, and cultures of Ps. aeruginosa gave fewer colonies per spoon for the same viable count.

2. The surface tension of cultures of E. coli and Staph. aureus were not significantly different, and it was concluded that surface tension was not an important factor in the difference in the relation between the spoon count and the viable count for these two species.

3. Cultures of Staph. aureus were subjected to ultrasonic waves to disrupt the clumps of cocci. It was found that after this treatment the relation between the viable count and the spoon count was the same as for the other organisms investigated except Ps. aeruginosa. The unique position of the latter organism was not investigated.

4. Alteration in the pH of a culture, and substitution of one medium for another in the spoon resulted in considerable changes in the relation between the spoon and viable counts. It is concluded that the surface charges on bacteria and on the medium are important in causing the bacteria to adhere to the surface of the agar as it is withdrawn from the medium. The viable counts of urines which are either strongly acid or alkaline are likely to be under-estimated.

5. Some minor changes in the dip-inoculum outfit which was described by Mackey and Sandys (1965) have been proposed, but these have largely been superseded by commercial preparations.

6. About 3000 dip-inoculum spoons were tested in the laboratory, and in an antenatal clinic, and were found to be sufficiently accurate for routine use, and to be of value if difficulties arise between the collection of the specimen and its reception in the laboratory.

7. The inoculation of the spoon by passing it through the uninterrupted stream of urine during micturition was studied as an alternative to dip-inoculation. About 1000 spoons have been tested in this way.

The use of the dip-inoculum spoon and the use of boric acid as a urine preservative are both discussed and compared. Each has deficiencies. The stream-inoculum spoon eliminates the principal drawbacks of both, possesses a major advantage in the simplicity and ease with which a mid-stream portion of the urine can be sampled, and carries only a slight penalty of a theoretical, (but unsubstantiated) reduction in accuracy.

The bacteriological and economic implications of the use of some of the commercially available spoons are discussed. These are probably cheaper than outfits prepared in the laboratory, but it is considered that some are un-necessarily complex (and expensive), and some are too large.

SECTION III

A trial of the use of oral penicillin G in the treatment of urinary tract infection

INTRODUCTION

With evidence marshalled from the literature and the laboratory bench indicating that penicillin G taken orally might be a useful urinary antibiotic, a clinical trial was planned. Obviously oral penicillin G in moderate doses presented no problems of toxicity that were not already well documented, but its use for Gram-negative infections of the urinary tract was almost an heretical proposition, and so it was necessary and desirable to carry out preliminary investigations on isolated patients.

The first part of this section comprises a report on the results of treating a few patients who had undergone gynecological operations and had post-operative bacteriuria. Subsequently it was possible to treat two patients with uncomplicated urinary tract infection with penicillin, and to follow the progress of these patients carefully, and finally the clinical trial was carried out with the aid of a number of general practitioners.

PRELIMINARY TRIALS

Plan of the trials

In order to gain confidence in the use of penicillin for urinary tract infection, and to get evidence with which it was hoped to persuade general practitioners that a full scale clinical trial was justified, the cooperation of Dr Garden H. Swapp, gynecologist at Woodend hospital, Aberdeen, was sought. It was agreed that a few patients with urinary tract infections following pelvic floor repair operations should have a course of 'Crystapen G', 500 mg six hourly in a short trial.

In fact none of the patients was properly followed up, or even supervised after the first few days of treatment, and so no conclusions could be drawn about the usefullness of penicillin under these circumstances. However the immediate response to the drug was recorded and is worth noting.

No interference was made in the management of the patients, save in the use of penicillin. Each specimen was taken by the nursing staff on the instruction of the medical staff in attendance, and went through the normal routine procedures in the bacteriology laboratory. These involved testing any isolates for sensitivity to streptomycin, tetracycline, ampicillin, kanamycin, colomycin, nitrofurantoin, nalidixic acid and sulphonamide by disk-diffusion. Estimates of penicillin sensitivity by tube-dilution and estimates of the quantity of penicillin in the urine were carried out as detailed in Section 1, pages 80 and 86.

Case No. 1. Mrs E.C., aged 43 years

After a pelvic floor repair operation, routine bacteriological testing of the urine indicated the presence of an asymptomatic bacteriuria. Five catheter specimens of urine, and one mid-stream specimen taken over a period of 9 days, all gave viable counts of more than 100,000 bacteria per ml of urine, the organism being a strain of E. coli fully sensitive to all the antibiotics against which it was tested by disk-diffusion. A tube-dilution sensitivity test against penicillin G was carried out and, the organism was found to be inhibited by 50 µg per ml.

On the first day after the start of penicillin therapy a mid-stream specimen of urine was sterile, and was found to contain 160 µg of penicillin per ml. A further specimen after 5 days of treatment was also sterile. The patient was then discharged from hospital, and no further follow-up was possible.

Case No. 2. Mrs B.A., aged 55 years

After this patient's operation an asymptomatic bacteriuria was diagnosed as result of the routine submission of specimens of urine to the bacteriology laboratory. Four out of five catheter specimens of urine taken post operatively contained more than 10^5 bacilli per ml, the fifth culture, being third in the series had less than 10,000 bacilli per ml. The organism (a strain of E. coli) was sensitive to all the antibiotics against which it was tested by disk-diffusion, and it was inhibited by 50 µg penicillin per ml.

On the day after penicillin therapy was begun a mid-stream specimen of urine yielded less than 10,000 E. coli per ml. She was, however discharged the following day, and a follow-up was not possible.

Case No. 3. Mrs F.R., aged 43 years

A catheter specimen of urine taken at operation was sterile, but after the operation the patient developed a large residual volume (300 ml) which became infected with E. coli. The strain was sensitive to all antibiotics against which it was tested except colomycin, to which it was resistant, and it was sensitive to penicillin (25 µg per ml) in the tube-dilution test.

One day after penicillin therapy was started a catheter specimen of urine yielded only small numbers of E. coli (less than 10,000 per ml), and catheter specimens on the fourth and fifth days of treatment were sterile, that on the fifth containing 40 µg penicillin per ml. On the eighth day the culture of a mid-stream specimen of urine yielded more than 10,000 but less than 100,000 E. coli per ml. Unfortunately the patient was discharged the following day and was not followed up.

Case No. 4. Mrs A.B., aged 40 years

After a pelvic floor repair operation at which urine culture was negative, this patient developed a urinary tract infection caused by a strain of E. coli sensitive to the eight antibiotics against which it was tested by disk-diffusion. The strain was sensitive to 25 µg per ml of penicillin by tube-dilution. After three days of penicillin therapy however culture yielded a strain of E. coli that was resistant to 300 µg penicillin per ml, and penicillin was abandoned in favour of nalidixic acid.

THREE CASE REPORTS

Introduction

The preliminary trials showed that penicillin did have a profound effect on the viable count in the urine of patients with symptomatic or asymptomatic urinary tract infection, but sufficient information was not available for valid conclusions to be drawn. Fortunately it was possible for the author to treat personally two patients with urinary infection, and to follow the cases in detail.

Case No. 1

This was a married woman of 28, who had two children but was not pregnant. She had a history of repeated urinary tract infections requiring hospitalisation on one occasion. Hospital treatment was followed by a period of nearly two years on suppressive antibiotic therapy (cycloserine, 250 mg on alternate days). For the past two or three years, however, she was symptom-free and was not taking antibiotics.

Clinical details

She had been well until two days before the diagnosis of urinary tract infection was made, when she became 'unwell'; she felt tired and noticed that her urine had an unpleasant odour. The next day she was much worse generally, with progressive abdominal pain, a sore back, and painful legs. She was extremly tired and 'washed-out'. The urine at this time was cloudy, she was incontinent and there was some dysuria. On this day (Day: -1) two mid-stream specimens of urine were collected, at 08:30 and 20:00 hours.

On Day 1 (the day on which treatment started) there was a rapid progress of symptoms, with urgency, frequency, dysuria, and haematuria at the end of micturition. Due to her family responsibilities she was not confined to bed,

although she took as much rest as possible. A further mid-stream specimen of urine was collected at 08:40 hours, and a final pre-treatment specimen at 17:40 hours when treatment with a single oral dose of 500 mg potassium penicillin G ("Crystapen G") was given. The timing of the dose relative to meals was not important as the patient had had nothing to eat that day.

Samples collected

After the single dose of penicillin, and for the next 50 hours, a mid-stream aliquot of every specimen of urine passed, with the exception of the first, was collected. The whole specimen was not collected, and so its volume could not be measured.

Samples of urine that were not dealt with within one hour were refrigerated. On each specimen, except the first two pre-treatment specimens, a viable count was performed by the technique of Miles and Misra (Miles, Misra and Irwin, 1938), and a portion of the un-spun urine was examined for pus cells and red blood cells microscopically. The quantity of penicillin in five of the first six post-treatment specimens was measured by the method described on page 80.

Bacteriological results

The results of the analysis of these specimens of urine are recorded in Table 45. In each of the four specimens taken off before therapy began there was a pure growth of a single strain of E. coli. The semi-quantitative methods (stream-inoculum spoon) used to examine the first two specimens indicated that there were more than 100,000 organisms per ml, and the more precise Miles and Misra technique showed that the subsequent two specimens had between 10^7 and 10^8 organisms per ml. There were, also, more than 10pus cells and more than 10 red blood cells per high power field in both the specimens examined microscopically.

TABLE 45

Results of the bacteriological investigation in Case No. 1

Number of specimen, day, and time specimen was obtained			Microscopic examination: number per high power field (1/6th inch) of		Viable Count of *E. coli* per ml	*Quantity of penicillin (μg) per ml of urine
No.	day	time	pus cells	red blood cells		
-4	-1	08:30	NT	NT	Over 10^5	NT
-3	-1	20:00	NT	NT	Over 10^5	NT
-2	1	08:40	Over 10	Over 10	6.08×10^7	NT
-1	1	17:40	Over 10	Over 10	2.72×10^7	NT
		500 mg of potassium penicillin G given by mouth				
1	1	18:10	NT	NT	NT	NT
2	1	18:50	3 - 10	3 - 10	4.16×10^3	256
3	1	19:40	1 - 3	1 - 3	768	128
4	1	20:40	3 - 10	Over 10	32	32
5	1	22:45	3 - 10	1 - 3	40	64
6	2	08:40	3 - 10	1 - 3	5.28×10^6	8
7	2	10:45	3 - 10	Nil	6.40×10^6	NT
8	2	13:15	1 - 3	Nil	9.60×10^4	NT
9	2	14:40	Nil	Nil	5.44×10^3	NT
10	2	22:00	Nil	Nil	4.88×10^7	NT
11	3	00:15	Nil	Nil	7.84×10^4	NT
12	3	08:00	Nil	Nil	7.43×10^6	NT
13	3	10:15	1 - 3	Nil	3.60×10^5	NT
14	3	11:50	1 - 3	Nil	6.48×10^5	NT
15	3	13:30	1 - 3	Nil	5.04×10^5	NT
16	3	16:15	Nil	Nil	9.28×10^6	NT
17	3	19:00	1 - 3	Nil	8.96×10^6	NT

NT = Not tested

* The results in this column have already appeared in Table 16.

Clinical and bacteriological progress

One hour after the single dose of penicillin there was a mild regression of symptoms, but after 5 hours there was still some urgency, though this was reduced. The patient was tired, and low back pain persisted. During this period the specimens collected showed a considerable reduction in the number of pus cells and red blood cells, and the viable count fell to 32 bacilli per ml, but fresh blood was passed at the end of micturition on each occasion during this first five hour period, though not subsequently.

The next day the number of organisms per ml of urine returned nearly to the level existing before treatment started, but symptomatically the patient improved steadily. By that evening, although there was still some slight dysuria and a little back pain, there was no abdominal pain, and she was feeling much better and not so tired. Moreover, the pus cell count declined to zero, there was no blood in the urine and no fresh blood was passed at the end of micturition.

On the morning of the third day, 36 hours after taking penicillin, the symptomatic improvement was maintained, but in the afternoon and evening her condition relapsed rapidly with a persistent mild dysuria and back pain becoming rapidly more severe, and accompanied by loin pain, frequency, urgency and stranguary. There was not however, any blood in the urine, although there were some pus cells. By the evening of the third day, 50 hours after the dose of penicillin, further treatment could be delayed no longer.

Further bacteriological details

The quantities of penicillin in all specimens of urine except the first passed during the first 15 hours after the dose of penicillin are shown in Table 45. The specimens of urine were refrigerated after collection, and some hours after

the last one was passed they were individually filtered through millipore filters (pore size 0.45 μm), and then deep frozen at -20°C for several days until it was convenient to test for penicillin. When carried out, the tests were performed as detailed in Section 1, page 92.

Case No. 2

Case No. 2 is a continuation of Case No. 1. With the recurrence of symptoms and the persisting bacteriuria a regular course of potassium penicillin G ('Crystapen G') 500 mg four times per day, was commenced at 20:00 hours, 50 hours and 20 minutes after the first dose had been given.

Clinical response

After the institution of a regular course of penicillin therapy the clinical condition of the patient, far from improving rapidly as it had done after the first dose, became steadily worse, so that by midnight (4 hours after the first dose) all the symptoms of a severe acute urinary infection were present, but there was no haematuria. Because of the delay in the response to treatment compared with the first occasion, and because it was thought possible that a penicillin-resistant organism might have emerged, a change of drug was considered, but it was finally decided to await the morning and the results of the examination of the latest specimens before instituting a change, so that a new antibiotic could be chosen in the light of the most recent antibiotic sensitivity tests.

However by the following morning the symptoms had subsided considerably, and the sensitivity tests indicated that the organism had not changed. The regime was therefore continued. After this, improvement though slower than expected, was nevertheless steady, so that by the third day of treatment the only residual complaints were a continuing low back pain and tiredness, neither

of which were severe. After nearly four weeks of treatment, in the latter part of which the patient had become lax about taking the drug regularly, penicillin therapy was stopped. Follow-up specimens taken after two days and after three and six weeks were sterile.

Bacteriological results

The bacteriological results of this case report are summarised in Table 46. In no specimen was any blood noted on microscopy, nor was there any fresh blood passed at the end of micturition, but it took some 48 hours to eliminate the pus from the urine. The viable count fell rapidly so that in the first morning specimen after treatment there was only 64 organisms per ml, and this was only exceeded once thereafter. Small numbers of bacilli persisted in the urine for some 36 hours until sterile urines were obtained. After this, small numbers of E. coli (80 per ml) and Str. faecalis (296 per ml) were obtained from one sample of urine only, and there were considered to be contaminants from a carelessly taken specimen.

In the first 48 hours after penicillin therapy commenced the patient passed urine on 11 occasions, and a mid-stream aliquot was obtained from each one. After this, occasional specimens only were tested until penicillin therapy was stopped. As tests of cure, a specimen was collected two days after treatment stopped, another after three weeks and a final sample after six weeks. In none of these samples was any organism isolated.

Antibiotic sensitivity of the isolates

The antibiotic sensitivity of each isolate from Case No. 1 and Case No. 2 was tested by disk-diffusion. The Oxoid multodisk (see page 89) was used, and the disk containing 100 µg of penicillin. All the strains of E. coli were sensitive to penicillin and to the other antibiotics. The strains of Streptococcus

TABLE 46

Results of the bacteriological investigations in Case No. 2

Number of specimen, day, and time specimen was obtained			Number of pus cells per high power field	Species of organism isolated	Viable count of bacteria per ml
Number	day	time			
-2*	1	16:15	Nil	E. coli	9.28×10^6
-1*	1	19:00	1 - 3	E. coli	8.96×10^6
			Commenced penicillin G therapy at 20:00 hours		
1	1	21:30	3 - 10	E. coli	7.68×10^3
2	1	23:50	3 - 10	E. coli	3.54×10^3
3	2	00:30	Over 10	E. coli Str. faecalis	1.04×10^3 1.12×10^3
4	2	09:30	Over 10	E. coli	64
5	2	10:30	Nil	E. coli	184
6	2	15:00	1 - 3	E. coli	40
7	2	19:40	1 - 3	E. coli	32
8	2	23:50	Nil	E. coli	16
9	3	09:45	Nil	E. coli	56
10	3	15:00	Nil	Nil	sterile
11	3	17:45	1 - 3	Nil	sterile
12 - 15	4 - 9	-	Nil	Nil	sterile
16	23	-	Nil	E. coli Str. faecalis	80 296
17	24	-	Nil	Nil	sterile
			Penicillin therapy stopped on the 27th day		
18 - 20	29 - 72	-	Nil	Nil	sterile

* These specimens have already been recorded in Table 45.

faecalis were both sensitive to penicillin, ampicillin, nitrofurantoin and colomycin, but resistant to nalidixic acid, kanamycin, streptomycin, sulphonamide and tetracycline. The strain of E. coli isolated immediately before the first single dose of penicillin, and the strain isolated before the course of treatment began, and one chosen at random from those isolated on the second day of the course of treatment (from specimen No. 5) were tested by tube-dilution. Each was sensitive to 25 µg of penicillin per ml.

Case No. 3

This was a female child aged 2 years and 9 months who, over a period of 12 hours, developed frequency and dysuria, the latter to such an extent that there was near panic at the prospect of micturition. The child was not pyrexial, but was quiet, and 'not herself'. There was a history of difficulty in toilet training, but urine samples taken previously to investigate this problem had been negative. Two mid-stream specimens were obtained during the day. Microscopy of these specimens (unspun) showed large numbers of pus cells and motile bacilli in both, but no blood.

Treatment with penicillin was started before the results of the culture, of the first specimen were available. One hundred and twenty five mg of potassium penicillin G were given six hourly. One half of a crushed tablet of "Crystapen G" in jam was used at first, but there was considerable difficulty in getting the child to take this, so 'Crystapen G' syrup was tried with even less success for a few doses before returning to the tablet preparation.

Clinical progress

Treatment was begun at 19:00 hours, and within an hour the child was much better and was sleeping peacefully. She passed urine at 9 p.m., and although

TABLE 47

Results of the bacteriological investigations in Case No. 3

Number of specimen, day, and time specimen was obtained			Number of pus cells per high power field	Viable count of bacteria per ml
Number	Day	Time		
-2	1	13:15	over 10	2.30×10^6
-1	1	19:00	over 10	7.12×10^5
	Commenced penicillin G therapy at 19.00 hours			
1	2	21:00	Nil	sterile
2	3	-	Nil	sterile
	Treatment altered to 250 mg twice daily on 3rd day			
3	5	-	Nil	sterile
	Treatment stopped on 5th day			
4	7	-	Nil	sterile
5	11	-	Nil	sterile
6	27	-	Nil	sterile

there was considerable fear at the prospect of micturition, there appeared to be little discomfort. There was no discomfort and little fear when she passed urine at 10.00 p.m. Clinical recovery was dramatic and appeared complete in 36 hours. On the third day because of difficulty in getting the child to take the penicillin the regime was altered to 250 mg twice daily, and finally stopped on the fifth day because of continuing difficulty, and in view of the obvious health and vigour of the child.

Bacteriological results

The results of the bacteriological investigations are shown in Table 47. The two pre-treatment specimens both yielded a pure strain of E. coli which was

sensitive to the eight antibiotics against which it was tested by disk-diffusion (see page 198), and to 25 µg per ml of penicillin in a tube-dilution test.

Three specimens were taken during treatment and three after treatment had been stopped, and all were sterile. The child's recovery was complete clinically and bacteriologically.

MAIN CLINICAL TRIAL

Introduction

The last link in the chain of evidence for the value of oral penicillin G in the treatment of Gram-negative urinary tract infection was a large clinical trial of the drug comparing it with established urinary antibacterial agents. General practitioners were approached and asked to cooperate in the trial, which eventually involved 15 practitioners from 6 practices and 530 patients in the City and County of Aberdeen.

Plan of the trial

Because it is generally considered that penicillin G given by mouth is valueless for the treatment of Gram-negative urinary tract infection, the author judged it impertinent, in the early stages of the trial, to ask a practitioner to cooperate if he did not know what drug was being prescribed, and from which he could not select-out patients and treat them with other drugs if he so desired. It was considered unethical to use a placebo.

The principal details of the trial were first agreed between the author and three partners in a practice in Aberdeen. They were as follows -

1. The stream-inoculum outfit was to be used by the practitioners for all cases where urinary tract infection was suspected.

2. Those patients whom the practitioner would, in normal circumstances, have treated with sulphamethoxazole, which was the antibiotic of first choice for urinary tract infections in this practice were to be treated with penicillin G if the surname began with the letters A to M, or with sulphamethoxazole if the name began with the letters N - Z. This crude method of random selection was used because it was fundamental to the success of the trial that it should be kept simple, since the practitioners themselves

were not deeply concerned about the trial, and complicated rules might have been forgotten. Secondly, this method of selection was likely to give a slightly larger group of patients treated with penicillin than that treated with sulphonamide.

3. The dosage of penicillin was normally to be 500 mg six hourly for 10 - 14 days, and that of sulphamethoxazole 1 G loading dose and 500 mg six hourly thereafter for a similar period. The practitioners were free to alter this schedule if they chose, and clearly did so for children.

4. Only one pre-treatment specimen was to be taken, and treatment was to start immediately thereafter. The existence of only one specimen taken before treatment is regrettable, but in general practice where the doctor and, the patient are used to dealing with the situation without taking any specimens, and to prescribing and starting treatment with a minimum of delay, it is not possible to insist that treatment be deferred until a second specimen be obtained.

5. The practitioners were to be free to with-hold treatment from patients if they considered that a urinary tract infection was unlikely, or to treat with any other antibiotic if, for any reason they considered that this was desirable.

6. The practitioners were to inform the author if treatment was changed, but in fact this occurred only rarely. However the author checked the practice notes of all patients where this may have been done.

7. During the third week after treatment had begun (whatever that treatment was) a second stream-inoculum spoon was to be posted directly to the home address of the patient, who was to inoculate and return it. The results of this specimen were to be posted to the practitioner concerned.

Similarly in the seventh week a third specimen was to be sent to the patient and reported as above. Later this procedure was modified so that patients whose first two specimens were negative were excluded from the third specimen. Copies of the letters accompanying these specimens are included in the Appendix II. The practitioners were to explain how the spoon should be inoculated at the first visit, and further instructions were not to be given unless a previous sample from that patient had been inoculated incorrectly.

Modifications made while the trial was in progress

As the trial progressed the author approached other practitioners so that eventually 6 practices and 15 practitioners were involved. Some of the doctors were sceptical about the use of sulphonamide as the alternative to penicillin, and no pressure was exerted to force its use. In one practice nitrofurantoin was the established first line of treatment, whilst in another, which submitted only a few specimens, tetracycline was the drug used initially. In such circumstances it was considered adequate to treat the control group with the antibiotic normally used by the practitioner concerned.

Other modifications were made as the trial progressed. The request form was altered to encourage the recording of the marital state of the patient and eventually a short questionaire was included with the specimen sent out during the third week with the purpose of:

(1) making a superficial clinical assessment of the effect of treatment,

(2) assessing the number of patients who did not take the tablets, and

(3) encouraging patients to report toxic reactions, changes of treatment, or any other unforseen circumstance.

Copies of both forms and the questionaire are included in Appendix II.

Preparation and distribution of the stream-inoculum outfits

The stream-inoculum spoons were prepared in the laboratory by the method described in Section II page 168. The prepared outfit was packed in a suitable box and then if it was to be sent to a general practitioner, it was placed, with a request form in an open pre-paid reply envelope, and a box of these were delivered to each practitioner. The practitioner could then take an envelope from the box, and in it was all that was required for inoculating and sending the specimen to the laboratory.

When the outfit was to be sent to the patient at his home for a follow-up examination it was packed with a letter explaining why the specimen was sent (although this had been explained to the patient by the doctor) and asking the patient to inoculate the spoon "in the same way" and return it in the envelope provided. There is a copy of the letter in Appendix II.

Procedures carried out on receipt of the inoculated spoon

On receipt of the stream-inoculum outfit it was examined carefully for the presence of free urine (i.e. not absorbed in the foam wad). If this was present it was cultured on blood agar and MacConkey agar, and a semi-quantitative estimate of the number of bacteria present was made (McGeachie and Kennedy, 1963). If the specimen was the first from the patient and the patient had been put on antibacterial treatment that patient was eliminated from the trial although the specimen was reported and followed up for the information of the practitioner.

If the specimen was a follow-up examination a further stream inoculum outfit was at once sent to the patient with instructions on the correct method of inoculating it. On receipt of a correctly inoculated spoon the results of the culture of the urine were discarded.

Identification of pathogens

Following the initial examination of the spoon, the specimen was incubated overnight at 37°C. The following morning it was examined and, if there were less than 5 colonies (11 in the case of colonies of Staph. aureus) it was discarded and counted as negative. Where the colonies were confluent or semi-confluent a representative area of the spoon was subcultured on blood agar and MacConkey agar and incubated overnight to get discrete colonies.

Infecting organisms were identified according to the regime detailed in Table 4. A single colony was also subcultured on to a Dorset's egg slope for storage.

Antibiotic sensitivity

A single representative colony of each pathogen was picked either from the spoon or from the subculture on blood agar and plated on to Oxoid diagnostic sensitivity test agar. An Oxoid Multodisk (see Table 5) was laid on the plate. A single disk containing 100 µg of penicillin G (prepared by Oxoid Ltd) was also laid on the plate which was allowed to lie on the bench for at least one hour to allow pre-diffusion to take place.

The Oxoid diagnostic sensitivity test agar plates were prepared approximately once a week, and stored in a refrigerator. Each batch was tested with a penicillin- and sulphonamide-resistant strain of E. coli and a penicillin- and sulphonamide-sensitive strain of the same species. No batch had to be discarded.

A zone diameter of twice the diameter of the penicillin disk (14 mm) was the criterion for sensitivity. If the inoculum was heavy the presence of a fine growth within a well defined zone around the sulphonamide disk was not considered to indicate resistance, and bacteria showing this phenomenon

were reported as sensitive to sulphonamide.

Controlled trial

The worst feature of the organisation of the trial as recorded so far, was that if a patient was suffering from a particularly severe urinary infection there might be a tendency on the part of the doctor to withdraw him or her from the trial if the surname began with A to M, so that the practitioner could use an antibiotic in which he had confidence, whereas if the surname began with the letters N - Z this tendency was not so marked. If this occurred it would tend to bias the penicillin group with mild infections compared with the control group.

To overcome this a controlled trial was designed in which neither the author, nor the practitioners knew which drug was being used to treat the patient. Identical tablets containing either 250 mg of potassium penicillin G, or 250 mg of sulphamethoxazole were obtained. These were bottled and labelled "The treatment" and "The tablets" respectively, but only the pharmacist knew which drug was in each bottle. When the stream inoculum outfits were made up for issue to the practitioners a randomly chosen bottle of tablets was included with each one.

When a practitioner issued a stream inoculum outfit to a patient, he automatically got a bottle of medicine to use, and the type of tablets in the bottle (either 'treatment' or 'tablets') was already recorded on the request form to be sent to the laboratory (see Appendix II). If there was any contra-indiction to the use of either penicillin G or sulphonamide, or should the practitioner wish to withdraw a patient from the trial for any other reason, he could still do so, and substitute any other treatment, or none: but there could be no further bias in favour of one or other of the two groups.

Use of a computer

The clinical findings and results of treatment were coded and processed by the ICL computer at the University of Dundee. The programme related the clinical and bacteriological data, and is reproduced in full in Appendix I.

RESULTS OF THE MAIN CLINICAL TRIAL

Age, Sex and Marital state of the patients

There were in all 530 cases, of which 75 were male and 455 (86 per cent.) were female. The age and sex distribution is shown in Table 48, and it can be seen that whilst the numbers of presenting cases in each age group was steady among the males, (with the under 6's and the 56 -65 age group showing a slight increase in numbers) more than a quarter of the female cases came from the 16 - 25 age group. This high proportion of cases was not associated with pregnancy, which accounted for only 21 of the 132 patients. Of the women of 16 years and over 77 per cent. were married, but in the 16 - 25 age groups the proportion was only 49 per cent.

TABLE 48

Age and sex distribution of the patients in the trial

Sex	Number	Number between the ages (years) of									
		0 - 5	6 - 15	16 - 25	26 - 35	36 - 45	46 - 55	56 - 65	66 - 75	76 - 85	Not known
Male	75	11	8	8	7	4	6	15	7	7	2
Female	455	26	21	132	69	60	48	54	32	9	4

Definition of infected urine

There were 502 specimens in which the viable count of bacteria per ml exceeded 10^4, excluding a few patients with growths of Staph. albus which were considered to be contaminants. Of the 502 specimens 47 had less than

10^5 bacteria per ml, and this represented 10.5 per cent. of all first specimens, and 8.2 per cent. of subsequent specimens. In clinical practice a decision on the significance of each specimen would be made taking into account all the bacteriological and clinical factors, and confirmatory specimens might be requested. In this survey, because of the method of taking the specimen (with a stream-inoculum spoon) it was decided to include within the definition of significant bacteriuria all these specimens. An examination of Kass's report (1956) shows the patients with a diagnosis of pyelonephritis supplied the majority of specimens recording a viable count of between 10^4 and 10^5 bacteria per ml.

Proportion of cases with infected urine

Of the 530 patients the initial specimen of urine submitted by the patient indicated a viable count of less than 10^4 bacteria per ml in 272 cases (51 per cent.). A few of these cases may have been missed positive cases since only a single mid-stream specimen of urine was used as the criterion of diagnosis; a further number were patients for whom the practitioner was excluding urinary tract infection, rather than diagnosing it, and these were not treated; and the rest belong to that group which Gallagher called the 'urethral syndrome' (Gallagher et al., 1965).

Of the 258 patients with positive initial specimens, 16 did not submit a second specimen or were untreated, so there were only 242 patients for the treatment trial, 46 per cent. of the total.

The age distribution of these patients with a positive first specimen of urine is shown in Table 49. The low proportions of positive specimens among the 6 - 15 and the 26 - 45 age groups are notable because they are inconsistent with all the others which lie between 52 and 61 per cent.

TABLE 49

Proportion of the number of cases in each age group with a positive first specimen of urine

Age group	Number of cases with a positive first specimen of urine	Proportion of cases in that age group (%) with first specimen positive
0 - 5	21	52
6 - 15	10	35
16 - 25	73	53
26 - 35	27	36
36 - 45	23	36
46 - 55	33	61
56 - 65	41	59
66 - 75	21	57
76 - 85	9	56

Nor can this be ascribed to small numbers in these age groups because the 26 - 45 year group includes 140 patients.

False-negative specimens

There were 31 patients whose first stream-inoculum spoon recorded less than 10^4 bacteria per ml, but who submitted a second spoon in the third week with over 10^5 bacteria per ml. An examination of the clinical and bacteriological details of each case was made in order to assess how many of these 31 patients were in fact suffering from a urinary infection that had been missed by the pre-treatment specimen. The following details, which were considered to be the clinical criteria of infection, were noted:

(1) whether the third specimen (in the 7th week) was also positive,

(2) whether, in the light of the clinical situation, the practitioner had given a course of treatment following the positive (second) specimen.

(3) whether there was a history of urinary tract infection in the past, and

(4) whether haematuria was recorded on the request form that had accompanied the first (negative) specimen.

The third and fourth items were noted because these factors are shown (see Table 51) to be more frequently associated with the presence of infected urine than other signs and symptoms.

The results of this examination are compared with the results of a similar clinical and bacteriological examination of the details of 31 consecutive treated patients without bacteriuria in either the first or second specimens (representing a group of patients least likely to have urinary infection) and of 31 consecutive treated patients with positive first and second specimens (representing patients most likely to have infection). The results are shown in Table

It appears likely, from the results of Table 50 that a proportion of those patients submitting negative pre-treatment specimens were, in fact suffering from urinary tract infection. It is not possible to estimate accurately what that proportion was, or to speculate upon the number of patients with urinary infection who would have remained untreated had treatment awaited the results of bacteriology.

These false-negative specimens could have been caused by:

(1) absence of bacteria from the urine for any reason,

(2) failure of the stream-inoculum spoon to record the number of organisms per ml of urine correctly, or

(3) failure of the patient to inoculate the spoon correctly.

If the criterion of infection had been not one, but two consecutive mid-stream specimens of urine containing a significant bacteriuria, and if treatment had awaited the results of these examinations, there would have been few patients treated unnecessarily, but there may have been many suffering from urinary infection who would have been excluded from treatment.

TABLE 50

The incidence of false-negative specimens of urine: a comparison of the clinical and bacteriological details of 3 groups of patients

Bacteriological details of the patients all of whom were treated	Number of patients	Clinical details of the patients: number who fulfilled at least		
		1 of the criteria of infection	2 of the criteria of infection	3 of the criteria of infection
Patients with negative specimens before and after treatment	31	11	0	0
Patients with a negative specimen before treatment and bacteriuria subsequently	31	15	6	2
Patients with bacteriuria before and after treatment	31	24	11	4

False-positive specimens

In this investigation it has not been possible to trace false-positive specimens, or to make any estimate of their frequency. However, two

important causes of false-positive diagnosis have been eliminated, viz. the multiplication of small numbers of bacteria during transit of the specimen to the laboratory, and the spurious 'mid-stream' specimens of urine.

Signs and symptoms of urinary tract infection

An analysis of the principal signs and symptoms recorded by the general practitioners on the request chit is shown in Table 51, and the proportion of times that each symptom or sign was associated with an infected urine is shown.

TABLE 51

Relation of diagnostic signs and symptoms of urinary tract infection with the presence of an infected urine

Symptom or sign	Number of times recorded	Number (and %) of times symptom was associated with bacteriuria
Frequency of micturition	307	157 (51)
Dysuria	294	162 (55)
History of previous infection	134	95 (70)
Loin, supra-pubic, back or abdominal pain	129	41 (32)
Haematuria	39	25 (64)
Pregnancy	26	14 (54)
Urgency	24	12 (50)
Wetting	17	9 (53)
Pyrexia	13	4 (31)

With the exception of a history of previous urinary tract infection, and haematuria, none seem to be of much value in distinguishing true urinary infections from the urethral syndrome. In fact only one in three patients suffering from pain which was ascribed to the urinary tract, and one in three of those with pyrexia had infected urine.

TABLE 52

Relation of diagnostic signs and symptoms of urinary tract infection with the presence of an infected urine in children of 5 years and less

Symptom or sign	Number of times recorded	Number (and %) of times symptom was associated with bacteriuria
Frequency of micturition	11	10 (91)
Dysuria	11	6 (57)
History of previous infection	8	7 (88)
Wetting	10	7 (70)
Pyrexia	5	1 (20)

The relation of the signs and symptoms of urinary infection with the presence of an infected urine was calculated for each age group, but only in the 0 - 5 group was there any significant proportional differences from the figures recorded in Table 51. Table 52 shows that among young children frequency of micturition, ennuresis (either nocturnal, diurnal or unspecified) and a history of previous infection were all more reliable indicators of the presence of infected urine than was the case for older children and adults.

TABLE 53

Results of the clinical trial

Drug used in treatment	Number of patients	Number (%) of patients who provided negative urines in the third week	Number of patients who provided follow-up urines in the seventh week	Proportion (%) of follow-up patients that relapsed by the seventh week
Penicillin*	94	56 (60)	46	27
Sulphonamide*	59	27 (46)	23	0
Ampicillin	27	12 (44)	9	44
Nalidixic acid	15	10 (66)	5	0
Nitrofurantoin	27	18 (66)	14	35
Tetracycline	20	11 (55)	7	42
Total*	242	136 (56.2)	108	23.2
Double-blind trial				
Penicillin	30	15 (50)	12	33
Sulphonamide	29	12 (41)	12	0

* Includes cases treated with double-blind drugs

Clinical trial

The results of the clinical trial are tabulated in Table 53. Of the 242 patients analysed in the trial, 136 furnished a negative spoon (less than 10^4 bacteria per ml) in the third week, 56.2 per cent. of the total; 106 remained infected.

The only truly comparable groups of patients are the two given either penicillin or sulphonamide in the double blind trial. Although the numbers

are small, it can be readily seen that penicillin was effective in the treatment of this group of patients, and that a slightly higher proportion of the patients (the difference is not significant) who were treated with penicillin achieved a sterile urine after a two-week course of treatment, than was the case with sulphonamide.

It is not possible to compare the antibiotic groups in the uncontrolled trial uncritically because, for example, the high proportion of recurrent infections treated with nalidixic acid and tetracycline (see Table 54), and because of the possible selection, of mild cases for some antibiotics, such as penicillin.

However, some observations are justifiable. In the first place penicillin was used for 94 patients, 39 per cent. of all the cases in the trial, and it cleared the urinary tract of infection in 60 per cent. of these. The sulphonamide group, comprising 59 patients was the second largest group in the trial. The relatively poorer 'cure rates' for penicillin and sulphonamide in the controlled, double blind trial are discussed below, as are the indifferent results recorded when ampicillin or tetracycline were prescribed. Ampicillin cleared only 44 per cent. of patients of bacteriuria, and tetracycline only 55 per cent.

Relapse and re-infection

One hundred and eight (80 per cent.) of the patients who were negative in the third week submitted a further specimen for examination in the seventh week, and nearly a quarter of these (23.2 per cent.) showed a significant bacteriuria, including two specimens with between 10^4 and 10^5 bacteria per ml. These patients were assumed either to have relapsed, or to have become re-infected.

The most striking feature of the relapse rates is the complete absence of any proved relapse or re-infection among those patients treated with sulphonamide and nalidixic acid. Although the numbers, particularly of those treated with nalidixic acid are small, nevertheless, together these two groups comprise 26 per cent. of all those patients cleared of bacteriuria after treatment and followed up for 7 weeks. Furthermore even if all the patients cleared of bacteriuria in the sulphonamide group who did not submit a follow-up specimen had suffered a relapse (which is unlikely), the 'relapse rate' would have been only 15 per cent., much lower than the relapse rate following treatment with any other drug.

Since there was no pressure upon the patient to submit the follow-up spoon there is almost certainly some degree of bias in this study towards a higher than actual relapse and re-infection rate, because those who felt well would have been less likely to return the follow-up specimen than those whose treatment had been less successful.

Another notable feature of the follow-up was the high relapse rates among those treated with ampicillin, nitrofurantoin and tetracycline, which were 44, 35 and 42 per cent. respectively. These poor results can be due only in part to the selection of patients with recurrent infection for treatment with these drugs (see Table 54).

Significance of previous urinary tract infection

More than one third (37 per cent.) of the patients had a history of recent (and some of frequently recurrent) infection of the urinary tract. The results of the treatment given during the trial to this group of patients are compared with the results achieved among those patients with a first infection in Table 54. In all 61 per cent. of patients with a first

infection were cleared of bacteriuria after treatment, compared with 46 per cent. of patients with recurrent infection. Furthermore the relapse rate in the group with recurrent infection was more than twice as great as it was in the group with no known previous infection (39 per cent. and 18 per cent. respectively).

Double blind trial

Unfortunately all except two of the patients treated with sulphonamide had no history of previous infection. It is not possible therefore to compare the use of penicillin and sulphonamide for recurrent infections in the double blind trial. A comparison of penicillin and sulphonamide used for patients with no previous infection shows that penicillin was marginally better than sulphonamide in clearing the infection, but whilst nearly a quarter of the patients taking penicillin relapsed, none of those who had had sulphonamide did so. The net result therefore (without allowing for any bias resulting from those patients who did not submit a specimen in the seventh week) was than sulphonamide was marginally better than penicillin in giving a lasting cure.

Uncontrolled trial

It is clear from Table 54 that some drugs, notably nalidixic acid and tetracycline were usually reserved by the practitioners for use for patients with recurrent disease. In spite of the small numbers involved the relative success of nalidixic acid in producing a long lasting effect is in direct contrast with the short lived response achieved by tetracycline, ampicillin and nitrofurantoin. In fact of the 36 patients with recurrent infection treated with nitrofurantoin, ampicillin and tetracycline, only 12 supplied negative specimens in the third week. Of these, unfortunately, only 5

TABLE 54

The results of the treatment of two groups of patients

Drug used in treatment	Total no. of patients	Patients with no history of previous urinary infection			Patients with a history of previous urinary infection		
		Number (%) of patients treated with drug	Percentage of patients 'cured' in third week	Percentage of patients followed up who relapsed	Number (%) of patients treated with drug	Percentage of patients 'cured' in third week	Percentage of patients followed up who relapsed
Penicillin*	94	67 (71)	58	21	27 (29)	63	40
Sulphonamide*	59	45 (76)	51	0	14 (24)	29	0
Nalidixic acid	15	3 (20)	66	0	12 (80)	66	0
Ampicillin	27	15 (55)	66	37	12 (45)	16	100
Nitrofurantoin	27	18 (67)	83	30	9 (33)	33	100
Tetracycline	20	5 (25)	80	25	15 (75)	46	66
Total*	242	153 (63)	61	18	89 (37)	46	39
Double blind trial							
Penicillin	30	20 (67)	50	24	10 (33)	50	50
Sulphonamide	29	27 (93)	44	0	2 (7)	...	...

* Including cases treated with double blind drugs

submitted a follow-up specimen, and four of them showed a significant bacteriuria. The same three drugs were more successful when prescribed to patients without previous infection, but the relapse rate (for the three drugs together) for the 25 patients followed up for seven weeks was at 32 per cent.

much higher than the relapse or re-infection rate for penicillin G which was 21 per cent. (Comment has already been made about the absence of any proved relapse or re-infection among patients treated with sulphonamide or nalidixic acid).

Among patients treated with penicillin G there was no significant difference in the proportion submitting a negative specimen in the third week between those with a history of previous infection and those without. Patients with known previous infections, however, were twice as likely to relapse or become re-infected by the seventh week.

Sulphonamide, in the uncontrolled portion of the trial, was much less successful when given to patients with recurrent infection (29 per cent. cleared of bacteriuria in the third week) than when given to patients without previous infection when 51 per cent. of patients were 'cured'.

Relation of the age of the patients to the prevalence of recurrent infection and the results of treatment

In Table 55 the results of treatment have been related to the age of the patient and the prevalence of recurrent infection. Because the numbers were small in some of the ten-year age groups, the patients have been grouped instead into a childhood group (0 - 15 years), "child-bearing" group (16 - 35 years), a middle aged group (36 - 55 years), and an elderly group (56 - 85 years).

The prevalence of recurrent infections was steady in the first three groups, at approximately 33 per cent. but it rose to 50 per cent. for the patients aged 56 and over. The 'cure rate' at the third week was also steady at about 60 per cent. for the first three groups, but dropped to 39 per cent. for the fourth group. The proportion of relapses was however highest

(at 42 per cent.) for both the youngest and oldest groups, and was only 12 per cent. for the "child-bearing" group. Age is clearly, therefore an important factor, those acquiring a urinary infection either in childhood or after the age of 55 having a much poorer prognosis than those with urinary infection in middle life.

TABLE 55

Relation of the age of the patient to the prevalence of recurrent infection and the results of treatment

Age group (years)	Number of patients analysed	Percentage of patients with recurrent infection	Percentage of patients who submitted a negative specimen in the third week	Percentage of patients followed up who relapsed
0 - 15	27	33	59	42
16 - 35	90	36	62	12
36 - 55	51	29	57	25
56 - 85	74	50	39	42

The effect of treatment of the patients' symptoms

Two hundred and Fifty three patients answered the two questions which were submitted with the first follow-up specimen (see Appendix II). The question enquiring whether the patient took the medicine regularly, was answered in the affirmative by such a large majority that analysis is not

worth while. An analysis of the answers to the second question, "Has the treatment made you better", is shown in Tables 56 and 57. Only patients who were treated are included in these tables. In Table 56 the answers of those patients whose first specimens of urine were infected are analysed, and a subjective cure (the patient considering that he or she was better) is correlated with a bacteriological cure, which was the provision of a negative specimen of urine at the first follow-up.

TABLE 56

A correlation of the subjective and bacteriological results of treatment of 157 patients with infected urine

Subjective opinion of the patient	Number (and % of total) of patients	Bacteriological result:	
		'Cure'	'Failure'
'Cure'	134 (85)	74	60
'Failure'	23 (15)	2	21

It is clear from these results that there is no correlation between a subjective 'cure' and a bacteriological one, and that treatment frequently converts a symptomatic infection into an asymptomatic one, or at least relieves the more severe symptoms. On the other hand persisting symptoms were quite a reliable sign that the infection had not been eradicated.

In Table 57 a correlation is attempted between the subjective and bacteriological results following treatment when the initial specimen was not infected.

which was in this case sulphonamide. The other results of the questionaire are recorded in Table 58.

TABLE 58

An analysis of the opinion of 11 general practitioners who had used penicillin G for urinary infection of its relative value compared with other established drugs

Number of doctors who considered penicillin to be as good as		Number of doctors who considered penicillin to be poorer than	
antibiotic	number	antibiotic	number
sulphonamide	4	nalidixic acid	2
ampicillin	3	ampicillin	5
nitrofurantoin	1	nitrofurantoin	2
tetracycline	2		

There appears to be little correlation between the results recorded in this table and the bacteriological results of the clinical trial. The latter indicated, for example, that the 'cure rates' following ampicillin and nitrofurantoin were, in this trial at least, poor, and that sulphonamide, which has come out worst in the opinion of the practitioners was one of the more effective drugs.

DISCUSSION

Preliminary trials

The preliminary trials added little factual information to the knowledge of the value of penicillin G as a urinary antibiotic. However, in several cases well established bacteriuria was eradicated with oral penicillin, and this information was important at the time, and was used along with the results of the three cases that are reported in the second part of this section, when practitioners were approached and asked to cooperate in a clinical trial.

Case Reports

Cattell and his associates (1968) in the course of their studies of dilution of urine and wash-out caused by a high fluid intake and hourly voiding, note the effect of a single dose of 500 mg of ampicillin (unaccompanied by increased fluids) on the number of bacteria in the urine of a single patient infected with an ampicillin-sensitive strain of E. coli. The viable count of bacilli in the urine was reduced from 7×10^8 to 1×10^7 over two hours, followed by several hourly specimens with viable counts between 10^4 and 10^5 bacilli per ml, with a return the following morning to a count of between 10^8 and 10^9.

In the patient whose results form the first case report there was a similar, but more dramatic fall in the viable count from 2.7×10^7 to 4.16×10^3 in 70 minutes, and a further fall to 32 bacilli per ml 5 hours after the administration of the drug. By the following morning the viable count had risen again to more than 10^6 per ml, but the clinical effect of the single dose of penicillin was more long lasting. It is noteworthy however that on only one occasion during the 50 hours after the single dose

of penicillin did the viable count exceed that which had been present before the dose, and that the presence of blood in the urine was eradicated, and pus was eliminated from the urine for about 20 hours. Furthermore the patient was symptomatically greatly improved for approximately 42 hours after the dose of penicillin, in spite of being bacteriuric for most of this time.

An even more dramatic result followed the administration of penicillin to the child whose case history constitutes the third report. This child whose spirited resistance to the, admittedly bitter taste of penicillin (however disguised) resulted in a drastic reduction in both the frequency and duration of penicillin therapy, was symptomatically greatly improved within one hour of therapy, and bacteriologically the urine was sterile in 2 hours (the first specimen passed after therapy began), and this improvement was maintained even though therapy was interrupted and lasted for only 5 days. Although the stated dosage for this patient was high on a weight for weight basis the actual amount administered was never certain, and on some occasions a considerable proportion of the drug was probably not swallowed.

The response to penicillin therapy recorded in Case Report No. 2., was complicated by the previous single dose of penicillin. The viable count fell rapidly at first from 8.96×10^6 to 7.68×10^3 after 90 minutes of penicillin therapy, but although counts as low as 16 bacilli per ml were achieved on the second day a sterile urine was not obtained until the third day. Symptomatically also the patient was slow to respond, so slow in fact that a change of treatment was very nearly made. However after 12 hours, progress was steady, and continued without relapse until complete. This patient's treatment was continued for nearly four weeks because of her history of intractible urinary infection, and in view of the poor initial response to the course of penicillin tablets.

The three reports indicate the undoubted effectiveness of oral penicillin G in uncomplicated cases of urinary tract infection. They also show that a very short course of only 5 days of intermittent therapy may be sufficient, although such a course would not be advised except in unusual circumstances and then only if adequate facilities for testing the urine existed. These studies confirm Stamey's observations about the usefullness of penicillin G, and also his statement that when an antibiotic is effective, the urine is probably sterile within 24 hours in many cases, and always within 48 - 72 hours (Stamey et al., 1965). (In Stamey's investigations a sterile urine was one with less than 1 organism per ml).

The failure of the strains of E. coli isolated from Cases 1 and 2 to develope resistance to penicillin, considering the nature of, and response to the treatment, was noteworthy in the light of the investigations reported in Section I (page 135), of this thesis, and of Stamey's observations that penicillin resistance does develop in vivo.

The clinical trial

A trial involving hospitalised patients was considered but rejected. About a quarter of urinary pathogens from hospital sources in Aberdeen were resistant to penicillin (see Table 22), and it appeared that penicillin (or ampicillin) was one of the first drugs to which an organism became resistant in the hospital environment. Furthermore, the large number of persons supervising the patient's progress in hospital (nurses, junior and senior medical staff), and the fact that in hospital most urinary tract infection is secondary and not therefore of prime interest to the clinician in charge, mean that other factors will play the major part in determining the management and follow-up of the case.

It may be that penicillin has a place in the treatment of hospital urinary tract infection, especially where cumulation due to high dosages or poor renal function may put a patient at risk if more toxic antibiotics are used. Even with ampicillin the serum concentration of the drug and the incidence and severity of toxic reactions are related (Lee and Hill, 1968).

TABLE 59

The cost of urinary antimicrobial agents

Proprietary name	Adopted name	Daily dose	*Cost of 12½ days' treatment	
			£p	£sd
Gantrisin	Sulphafurazole	2 g	£0.80	£0:16/-
Crystapen G	Potassium penicillin G	2 g	£0.93½	£0:18/8
Ledermycin	Dimethylchlortetracycline	600mg	£1.71	£1:14/2
Bactrim/Septrin	Sulphamethoxaxole + trimethoprim	1920mg	£2.20	£2:4/-
Furadantin	Nitrofurantoin	400 mg	£2.35	£2:7/-
Penbritin	Ampicillin	2 g	£3.45	£3:9/-
Negram	Nalidixic acid	4 g	£3.60½	£3:12/1

* 12½ days' treatment is chosen because it represents 50 doses with most of the drugs, and because the drugs are priced in 50's and 100's.

The main advantages of penicillin appear to be its absence of toxicity and its cheapness, both properties being particularly important in treating a common illness in general practice, and especially so when a proportion of patients without infection are likely to be treated. Garrod (1966) quotes Damashek (1960) who was reviewing drug hypersensitivity reactions and wrote, "The tragic thing about these cases, most of whom died, is that the drug need

never have been given". It is the clinician's and the bacteriologist's obligation to use and advise the use of, well tried, non-toxic antibiotics if these are likely to be efficacious, and only to consider others when these have failed, or if sensitivity tests indicate that failure is likely.

The cost of penicillin therapy is compared with the cost of six other urinary antibiotics and chemotherapeutic agents commonly prescribed by general practitioners in Table 59. Proprietary preparations have been compared because some of the drugs are not available otherwise, and the information is taken from the Monthly index of medical specialities (MIMS), Volume 12, Number 5, September, 1970.

A trial of the use of penicillin for urinary tract infection in general practice was therefore devised. The guiding principles behind the planning of the trial were first that it should be simple and easy to manage. A 'chill on the bladder' is considered by many women to be almost a normal, if very uncomfortable part of married life, and most practitioners are satisfied with the therapy available which appears to relieve the symptoms of the disease. A complicated trial would not find favour with either practitioners or patients. Secondly, in return for the doctors' cooperation the author attempted to offer an improved service. This consisted of the use of the stream-inoculum spoon, and of the obtaining and reporting of follow-up specimens without involving the practitioner.

The results of the trial

The overall results of the clinical trial were poor compared with some other trials of the treatment of urinary infection in general practice. Thus Mond et al., (1965) reports on the treatment of 43 episodes of urinary infection with sulphonamides, and found that 83 per cent. of them were cured at the sixth week.

Williams et al. (1968) reviewed the cure rates after treatment of bacteriuria of pregnancy of five published series, and compared them with his own results. In all 779 patients were treated, 667 with a sulphonamide, 81 with nitrofurantoin and 31 with cycloserine, and there were in addition 30 patients treated with a placebo. Most of the cure rates were between 70 and 80 per cent., with the lowest being 60 and the highest 82 per cent. The cure rate with the placebo group was 23 per cent. Neither the drugs used, nor the duration of therapy seemed to influence the results of treatment.

However some other surveys of urinary infection have not had such successful results. Gallagher et al. (1965) had a cure rate of 64 per cent. after treatment (sulphonamide was used in most cases), Stansfeld (1966) who treated 350 children noted that while initial success was very high (92 per cent.) the relapse rate was more than 50 per cent. Ganguli (1966) found in a trial of sulphonamide and cycloserine for antenatal patients with bacteriuria that there was no difference between the drugs, and that about 75 per cent. were cured after 1 week, but in only 51 per cent. of cases was this maintained for the rest of pregnancy.

The result of this present trial has been a 'cure rate' of 56.2 per cent. in the third week, with a relapse or re-infection rate of 23.2 per cent. over the next four weeks.

The degree of cooperation received from the patients is important in assessing the results of the follow-up. In particular it would be useful to know how many patients completed the course of treatment. The results of the questionaire in which the patients were asked whether or not they had regularly taken the tablets prescribed resulted in an overwhelming majority (nearly 97 per cent.) replying in the affirmative, figures which all the

practitioners questioned. It may have been significant that the results of the controlled trial were poorer than the results with similar antibiotics in the un-controlled trial. Selection of patients to be treated with "reserve" antibiotics (nalidixic acid, tetracycline, ampicillin and nitrofurantoin) is probably an important factor because it may have excluded patients with intractible infections from the penicillin group, but not the sulphonamide group in the early part of the trial. Another factor may have been the unpleasant taste and odour of the tablets used in the controlled trial. These had been specially formulated so that the penicillin and sulphonamide tablets were indestinguishable, and they were not film or sugar coated. Perhaps fewer patients took these tablets regularly for that reason.

Age and a history of previous infection have been shown to have a profound effect on the proportion of patients 'cured'. Women in the sexually active age-group have the best prognosis, reflecting perhaps an 'easy-come-easy-go' type of infection where there is no underlying deformity, and the infection is associated either with trauma or with pregnancy. Among older adults and children urinary infection is more commonly associated with underlying deformity, either congenitial, or acquired as a result of child-birth or long-standing untreated urinary infection.

Results with penicillin

The proportion of patients treated with penicillin who were 'cured' was similar to the overall total, which included this group, and the proportion which relapsed was also similar. When the patients were divided into a 'first infection' group and a 'recurrent' group, those treated with penicillin in the 'first infection' group had similar 'cure' and relapse or re-infection rates as those treated with other antibiotics.

Among patients with recurrent infection, however, those treated with penicillin had a higher 'cure' rate than those treated with other antibiotics, although this was probably due to selection of patients to be treated with the 'reserve' antibiotics.

When the patients treated with penicillin are compared with those treated with sulphonamide, the 'cure' rate was consistently better with penicillin, but whereas the relapse and re-infection rate for all penicillin treated patients was 27 per cent., and 33 per cent. for penicillin treated patients in the double blind trial, no patients treated with sulphonamide were shown to suffer a relapse or re-infection.

The opinion of the practitioners of the value of penicillin was dissappointing. While none thought that penicillin was poorer than sulphonamide (and one considered it to be better), when comparing penicillin with other antibiotics the numerical balance opinion was that penicillin was poorer than nalidixic acid, nitrofurantoin or ampicillin, but as good as tetracycline. If penicillin were as good as, for example ampicillin, then a range of opinions might have been expected, including a number who considered it to be an improvement.

Relapse and re-infection

No attempt has been made to correlate the organism present at either of the two subsequent examinations with that present before treatment. Gruneberg (1969) has shown that urinary infections are most likely to be caused by the organisms which are commonly present in the bowel. It follows that the organisms that caused the original infection will usually be available to re-infect the patient, so that identity of the bacteria causing the original infection and that present at follow up does not prove a relapse.

Neither is the converse true. Stamey and his colleagues considered that

in some cases two organisms may be involved in causing a urinary infection, but that because one has a considerable numerical superiority, the other is missed. If the most numerous organism is eradicated, but not the other, a mistaken diagnosis of re-infection may be made when the correct conclusion would be either a relapse or failed treatment.

Proportion of cases with infected urine

The number of patients with symptoms of urinary infection, but without an infected urine (51 per cent.) was, in this series, very similar to that described by Mond and his colleagues (1965) and Steensberg et al. (1969). Of those patients who submitted a negative pre-treatment specimen, 31 submitted a positive specimen after treatment. A comparison of this group with a group of patients with definite infection who had failed treatment suggested that a number of them had in fact been suffering from urinary infection which had been missed. In the light of Kimmelsteil's observations that "Chronic pyelonephritis can be recognised much more often at autopsy than during life" (Kimmelsteil et al., 1961) it would seem that not enough attention is paid to this group of patients. It is common practice for urinary tract infection to be diagnosed only if two or more consecutive specimens of urine have a significant bacteriuria (Savage et al., 1969; Mond et al., 1970). Where this is necessary because of a screening procedure which depends on specimens being collected and dealt with under less than ideal conditions, a sensible precaution would be to keep under review those patients whose second specimen was negative, lest having been caught they be allowed to escape.

The 'urethral syndrome'

The existence of a group of patients without demonstrable urinary infection

but presenting with urinary symptoms was confirmed. The findings of this trial agreed with those of Mond et al. (1965) and Gallagher et al.(1965) with regard to the age distribution, clinical findings and in particular the relative scarcity of patients with haematuria in the group. Gallagher found that 28 per cent. developed bacteriuria if followed up, and this survey confirms this. It may be, however, that these patients are not part of the 'urethral syndrome' at all. Those with the real 'urethral syndrome' may not develop bacteriuria, as Murdoch (Murdoch et al., 1968) has claimed. In this survey there were 72 patients whose urine remained un-infected before and after treatment. It seems likely that these patients suffer from a definite clinical entity, which is not a urinary tract infection and which deserves more study.

Importance of a test-of-cure

The absence of any correlation between the subjective opinion of the patient that he or she was cured, and the bacteriological fact of a cure or a failure to cure may be compared with the continued or maintained clinical improvement of Case No. 2 who was clinically improved for many hours after bacteriuria had returned. It is obvious therefore that a general practitioner can have little knowledge of the actual success of treatment without an examination of the urine.

CONCLUSIONS

Penicillin G given orally is suitable for use as a broad spectrum antibiotic against Gram-negative urinary tract pathogens in domicilliary practice.

For precise treatment of urinary infection a pre-treatment specimen of urine (which may be sent already seeded on to a dip- or stream-inoculum spoon or slide) is necessary. More important, however, is a follow-up specimen which is essential if the practitioner is to know whether his patient has a persistent urinary infection. Most patients, regardless of the presence or absence of bacteriuria, will be asymptomatic or considerably improved at this stage, so that bacteriological failure may be masked by spurious clinical success.

There exists a group of patients who suffer from the 'urethral syndrome', whose urine is not infected, and for whom the treatment given empirically for urinary infection may be irrelevant. Since this group forms a substantial proportion of the total it behoves the clinician to use the least toxic and cheapest effective drug available unless there are definite reasons to do otherwise. It is claimed that oral penicillin G fulfils these criteria.

SUMMARY

1. Four patients with Gram-negative urinary infection following gynecological operation were treated with oral penicillin G. In three out of the four cases the immediate bacteriological response was favourable, but adequate follow up was not possible.

2. A patient with a well established Gram-negative urinary infection was given a single oral dose of penicillin G (500 mg), and the response to this dose was carefully followed clinically and bacteriologically for the next 50 hours. The viable count was reduced from 2.72×10^7 to 32 bacilli per ml in five hours before returning almost to pre-treatment levels. Symptomatic improvement was steady and persisted for many hours after the bacteriological effect of the dose of penicillin had subsided. Blood was eliminated from the urine and did not return during the 50 hours of follow-up, and the amount of pus excreted was greatly reduced.

3. After 50 hours the same patient was given a course of penicillin G (500 mg six hourly by mouth) for four weeks. Clinically and bacteriologically the response was slower than it had been to the original first dose, but after 12 hours clinical improvement was steady and uninterrupted. The viable count in the urine was reduced from 8.96×10^6 to 3.54×10^3 in five hours, and bacteria were eliminated from the urine by the third day. Recovery was complete.

4. A female child aged $2\frac{3}{4}$ years with a urinary infection due to a strain of E. coli sensitive to 25 µg of penicillin per ml was treated with a five day course of potassium penicillin G, 0.5 g daily in divided doses.

Clinical improvement was dramatic and permanent, a sterile urine was obtained 2 hours after commencing therapy. There was no relapse.

5. A clinical trial of the treatment of urinary infection in general practice was instituted. Five hundred and thirty patients were involved, of which 51 per cent. (272 patients) were not infected. Two hundred and forty two patients who were infected were treated and followed up. Of these 94 were treated with penicillin, 59 by sulphonamides, and the remainder by one of the following - ampicillin, nalidixic acid, nitrofurantoin andtetracycline. Thirty of the patients treated with penicillin and 29 of those treated with sulphonamide formed a sub-group of patients whose treatment was double blind, in that neither practitioner, nor the author (bacteriologist) knew which drug had been prescribed. Tests of cure were performed in the third week and the seventh week.

6. Sixty per cent. of the group treated with penicillin submitted a negative specimen in the third week compared with 56.2 per cent. of all patients (including those treated with penicillin). The relapse rate among penicillin-treated cases was 27 per cent., compared with an overall figure of 23.2 per cent. In the double blind trial 50 per cent. of those treated with penicillin were 'cured' in the third week, and 33 per cent. of these relapsed, compared with a 41 per cent. 'cure' rate for sulphonamide treated cases with no relapses.

7. Other observations on urinary infection in the community have been made. It is considered that the urethral syndrome is a distinct entity and merits further study.

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

Such evidence that exists for, or against, the use of penicillin G for the treatment of Gram-negative urinary tract infections is confounded by ambiguous references to penicillin concentrations in the urine, uncertainty about the importance of serum levels of antibiotics when treating pyelonephritis, and vague references to the possible value of penicillin in certain ill-defined spheres of Gram-negative bacteriuria.

Garrod and O'Grady, for example (1968, page 371), although they quote the report by Scowen, Badenoch and Shooter (1957) which has been reviewed already on page 72, nevertheless indicate that parenteral penicillin is essential, at least initially, for the successful treatment of Gram-negative urinary infections. They state (page 361) that penicillin concentrations attainable in the urine exceed 250 µg per ml, but neither the dose nor route is recorded, although intramuscular therapy is inferred, and it is not clear whether this is a peak or a mean concentration. Similarly, Barber and Waterworth (1964) state that the concentration of penicillin likely to be attained in the urine (after an unstated dose by an unstated route) is 100 units (60 µg) per ml. Finally Kass in 1955 wrote, "Although penicillin has been little used in the treatment of Gram-negative bacillary infection of the urinary tract, there were indications in vitro that it might be effective against certain strains of proteus" (my italics).

The present investigations have shown that the mean concentration of penicillin per ml of urine in six healthy subjects for six hours after a single oral dose of 500 mg of penicillin G was 245 µg per ml. Measurement of penicillin concentrations in patients undergoing treatment did not indicate any significant differences. If the penicillin had been given

intramuscularly the mean concentration would have been 1500-2000 µg per ml in the same subjects, with peak concentrations very much greater (Eagle and Newman, 1947). These facts of penicillin absorption and its excretion in the urine have been known for a quarter of a century, although the actual levels of penicillin in the urine have not before been stated comprehensively.

Furthermore it is clear from the investigations reported in the 1940's that are reviewed at the beginning of this work (page 41), and from the results of the estimation of the mean inhibitory concentrations of penicillin for over 900 Gram-negative bacilli reported in this thesis, that between 75 and 100 per cent. of urinary pathogens are sensitive to as little as 50 µg of penicillin per ml. And yet there have been only isolated reports of the use of oral penicillin for urinary infections (Peeney, 1947; Scowen et al., 1957; Stamey et al., 1965), and these have been reviewed on page 72.

Stamey's object in using penicillin orally was to demonstrate that urine concentrations of the antibiotic are of paramount importance in pyelonephritis, even in the presence of proven renal tissue involvement. This conclusion, although disputed by Chisholm (1968) and Cockett (1965) and denied by Garrod and O'Grady (1968, page 357) who write, "If the substance of the kidney is involved, a drug with a systemic action is imperative", have powerful experimental evidence behind them (Stamey et al., 1960, 1965), and they are tacitly accepted by many clinicans and clinical bacteriologists in the day-to-day task of treating urinary infections. Furthermore, there is no evidence that the clinical success of nalidixic acid or nitrofurantoin, neither of which reach bacteristatic levels in the blood or kidney lymph (see page 40), is limited to those infections that do not involve the kidney substance.

In Murdoch's (1968) large series of patients all of whom had chronic urinary infection, and most with abnormal pyelograms, there seems to be little difference between those treated with ampicillin which may achieve bacteristatic levels in the tissues, and those treated with cycloserine which will reach such levels only in the urine. The results of the treatment of patients reported in this thesis confirm Murdoch's findings, and patients, even those with chronic disease, who were treated with penicillin or nalidixic acid had similar or improved success rates compared with those treated with ampicillin or tetracycline.

Even after Stamey's investigations (1965) which were reported in this country by Vinnicombe (1966) there has been no report of a comprehensive attempt to evaluate the use of oral penicillin for urinary infection. The reason for this failure cannot be the absence of any potential advantage. No antibiotic is cheaper or less toxic, none has been so thoroughly investigated or commands more confidence from the medical and lay public. The reason may lie in the teaching of medical students that penicillin is a 'narrow spectrum' antibiotic so that its use for Gram-negative bacillary infections is "so contrary to accepted opinion" that it has not been considered worthwhile. Furthermore the most likely role for penicillin G is as a first line drug for use by general practitioners, whereas other antibiotics are usually applied in hospitals first where research facilities are concentrated. Finally it is unlikely that any initiative towards establishing penicillin as a useful urinary antibiotic will come from the commercial drug houses that have invested much money and enterprise in the manufacture of other, valuable but expensive, drugs for the same condition.

The purpose of this thesis, which is distinct from the detached objective

of the experiments which are reported, has been to establish the position of oral penicillin as a broad spectrum urinary antibiotic, a position that has been indicated in different ways by other investigators, but which has not been comprehensively explored. Reference has already been made to the relation between the urine concentrations of penicillin achieved after oral therapy and the sensitivity of Gram-negative bacteria to this drug. It has been shown, as predicted by Stamey that Gram-negative bacilli may develop permanent penicillinase-producing resistance, but there is little evidence that this is an important element in clinical practice. No increase in resistance to penicillin was noted in the strains infecting the patients examined intensively, nor in the strains used in the model bladder, even though the bacilli could be recovered quite easily from urine containing bactericidal amounts of penicillin.

Experiments with the model bladder were of a preliminary nature, but they suggested that further experiments in which 'ureteric urine' and antibiotic are added together in amounts that have been shown to be secreted in vivo may be valuable. The results reported in this thesis show that the action of penicillin may be stimulated by the dynamic conditions whereby the infected urine in the bladder is being constantly diluted with ureteric urine, and this confirms the observations by O'Grady and his associates in several papers (reviewed on page 49 et sec.). The results have also shown that penicillin may be bactericidal under these conditions even when its concentration falls below the minimum required for inhibition in conventional sensitivity tests.

Before a clinical trial could be undertaken it was necessary to evaluate a method for diagnosing bacteriuria in patients attending general practitioners.

The author considers that enough attention is not given to the collection of specimens of urine. In few papers are the techniques precisely described or the difficulties emphasized. Turner (1961) and Sleigh (1964) have shown that preparation of the perineum does not affect the viable count when specimens are collected in a busy clinic. Under these conditions it is postulated that the collection of a mid-stream aliquot of an uninterrupted stream will reduce the contamination to a minimum. With this method, and without any peri-urethral cleansing, urine specimens containing less than 8 bacilli per ml were collected repeatedly from the patients who were investigated intensively both during and after antibiotic therapy.

To overcome the difficulties of posting specimens of urine the dip-inoculum spoon was tested, and during the course of the investigations the inoculation of the spoon by passing it through an uninterrupted stream of urine was tried and then tested fully. The use of the stream-inoculum spoon facilitates the examination of the mid-stream aliquot of even a very small stream of urine.

The results of the clinical trial show conclusively that oral penicillin G can abolish the symptoms of Gram-negative urinary infection rapidly and permanently in individual patients, and the overall results indicate that the proportion of patients successfully treated, when judged bacteriologically, was not smaller than the proportions successfully treated by established urinary anti-microbial agents such as sulphonamide, ampicillin, nitrofurantoin and tetracycline.

The scarcity of penicillin in the 1940's, which justly enforced the narrow-spectrum application of penicillin, has long given way to abundance, so that now, even though more than two-thirds of the drug are wasted, penicillin is the cheapest, and in many ways the best broad-spectrum antibiotic for urinary infection.

APPENDICES

APPENDIX I

COMPUTER PROGRAMME

The computer programme used to analyse the bacteriological and clinical data in Sections I and III is produced overleaf. The language is COBOL which is a highly developed, problem-orientated language, suitable for the simple analysis of large amounts of data, and relatively easy to read and understand. This programme has been run on the I.C.L. computer installed at the University of Dundee. Modifications were made in the programme on nearly every occasion that it was run, but the basic format remained unchanged.

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001                 IDENTIFICATION DIVISION.
002                 PROGRAM-ID. HULBERT-2.
003                 ENVIRONMENT DIVISION.
004                 CONFIGURATION SECTION.
005                 SOURCE-COMPUTER. ICL-4130.
006                 OBJECT-COMPUTER. ICL-4130 SIZE 3200 WORDS.
007                 INPUT-OUTPUT SECTION.
008                 FILE-CONTROL.
009                     SELECT CARDIN-FILE ASSIGN TO CREADER 0.
010                     SELECT CARDOUT-FILE ASSIGN TO PRINTER 0.
011                 DATA DIVISION.
012                 FILE SECTION.
013                 FD CARDIN-FILE RECORDING MODE IS F, LABEL RECORDS ARE OMITTED.
014                     DATA RECORDS ARE SPECIMEN-REC. SPECIMEN-REPORT, HEADER.
015                     PATIENT-REC. PATIENT-REPORT.
016                 01 SPECIMEN-REC.
017                     02 MARKER-1 PICTURE 9.
018                     02 MARKER-2 PICTURE XX.
019                     02 MARKER-3 PICTURE 999.
020                     02 MARKER-4 PICTURE 9.
021                     02 FILLER PICTURE XX.
022                     02 NO-GRO PICTURE 9.
023                     02 FILLER PICTURE X.
024                     02 ORG-TYPE OCCURS 6 PICTURE 9.
025                     02 FILLER PICTURE XXX.
026                     02 DISC-SEN OCCURS 9 PICTURE 9.
027                     02 FILLER PICTURE XXX.
028                     02 THOUSANDS PICTURE 9.
029                     02 FILLER PICTURE X.
030                     02 UIB PICTURE 9.
031                     02 FILLER PICTURE X(5).
032                     02 MARKER-7 PICTURE 9.
033                       88 NO-ORG-DETAILS VALUE IS 1.
034                       88 ORG-DETAILS VALUE IS 0.
035                     02 FILLER PICTURE XX.
036                     02 SEROTYPE PICTURE 9.
037                     02 FILLER PICTURE XX.
038                     02 BIOCHEM PICTURE 9.
039                       88 TYPICAL VALUE IS 1.
040                       88 TYPICAL VALUE IS 0.
041                     02 BIO-DETAILS.
042                       03 CITRATE PICTURE 9.
043                       03 UREA PICTURE 9.
044                       03 INDOLE PICTURE 9.
045                         88 POS VALUE IS 1.
046                         88 NEG VALUE IS 0.
047                       02 FILLER PICTURE XX.
048                       02 AGGLUT PICTURE 9.
049                         88 AUTOAGG VALUE IS 1.
050                         88 NON-AUTO VALUE IS 0.
051/
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051                 02 FILLER PICTURE X(5).
052                 02 RECUR-MARKER PICTURE 9.
053                     88 RECURRENT VALUE IS 1.
054                     88 NON-RECURRENT VALUE IS 0.
055                 02 FILLER PICTURE X(21).
056             01 PATIENT-REC.
057                 02 FILLER PICTURE X(9).
058                 02 NAME PICTURE A(20).
059                 02 FILLER PICTURE X.
060                 02 PRACTICE PICTURE 9.
061                 02 FILLER PICTURE X.
062                 02 TREATMENT OCCURS 9 PICTURE 9.
063                 02 FILLER PICTURE XXX.
064                 02 AGE PICTURE 99.
065                 02 FILLER PICTURE X.
066                 02 SEX PICTURE 9.
067                     88 MALE VALUE IS 0.
068                     88 FEMALE VALUE IS 1.
069                 02 FILLER PICTURE X.
070                 02 MSW PICTURE 9.
071                     88 MARRIED VALUE IS 1.
072                     88 SINGLE VALUE IS 0.
073                     88 WIDOW VALUE IS 2.
074                     88 NOT-KNOWN VALUE IS 3.
075                 02 FILLER PICTURE XXX.
076                 02 CLIN-SUCCESS PICTURE 9.
077                 02 FILLER PICTURE X.
078                 02 MED-TAK PICTURE 9.
079                     88 YES VALUE IS 1.
080                     88 NON VALUE IS 0.
081                     88 NOT-KNOWN VALUE IS 3.
082                 02 FILLER PICTURE XXX.
083                 02 SYMPTOMS OCCURS 11 PICTURE 9.
084                 02 FILLER PICTURE X(10).
085             01 SPECIMEN-REPORT.
086                 02 FILLER PICTURE X.
087                 02 ORGAN-1 PICTURE A(6).
088                 02 ANTIBIOT OCCURS 9 PICTURE AAA.
089                 02 MARKER-6 PICTURE 9.
090                 02 FILLER PICTURE X(45).
091             01 HEADER.
092                 02 FILLER PICTURE X.
093                 02 MARKER-5 PICTURE 9.
094                 02 HEADER-IN.
095                     03 TITLE-1 PICTURE A(30).
096                     03 TOTAL PICTURE A(7).
097                     03 TITLE-2 PICTURE A(10).
098                     03 TITLE-3 PICTURE A(10).
099                     03 TITLE-4 PICTURE A(10).
100                     03 TITLE-5 PICTURE A(10).
101                 02 FILLER PICTURE X.
102/
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102                 01 PATIENT-REPORT.
103                    02 FILLER PICTURE X.
104                    02 PATIENT-IN.
105                       03 DRUG-NAME PICTURE A(6).
106                       03 SUCCESS PICTURE A(7),
107                       03 FAIL PICTURE A(4).
108                       03 PER-CENT PICTURE A(8).
109                       03 SUCCESS-6 PICTURE A(9).
110                       03 FAIL-6 PICTURE A(6).
111                       03 RELAPSE-PC PICTURE A(10).
112                       03 CURE-6-PC PICTURE A(9).
113                       03 CSFP PICTURE A(4).
114                    02 FILLER PICTURE X(16).
115                 FD CARDOUT-FILE RECORDING MODE IS F, LABEL RECORDS ARE OMITTED.
116                    DATA RECORDS ARE TOTS, PATIENT-TOTALS, TITLE.
117                 01 PATIENT-TOTALS.
118                    02 FILLER PICTURE X(4).
119                    02 FILLER PICTURE X.
120                    02 FILLER-Y PICTURE X(131).
121                    02 PAT-TOT REDEFINES FILLER-Y.
122                       03 DRUG-NAME PICTURE A(6).
123                       03 DRUG-TOT PICTURE ZZZ9.
124                       03 SUCCESS PICTURE A(10) JUSTIFIED RIGHT.
125                       03 NO-1 PICTURE ZZZ9.
126                       03 FAIL PICTURE A(7) JUSTIFIED RIGHT.
127                       03 NO-2 PICTURE ZZZ9.
128                       03 PER-CENT PICTURE A(11) JUSTIFIED RIGHT.
129                       03 NO-3 PICTURE ZZZ9.
130                       03 SUCCESS-6 PICTURE A(12) JUSTIFIED RIGHT.
131                       03 NO-5 PICTURE ZZZ9.
132                       03 FAIL-6 PICTURE A(10) JUSTIFIED RIGHT.
133                       03 NO-6 PICTURE ZZZ9.
134                       03 RELAPSE-PC PICTURE A(13) JUSTIFIED RIGHT.
135                       03 NO-4 PICTURE ZZZ9.
136                       03 CURE-6-PC PICTURE A(12) JUSTIFIED RIGHT.
137                       03 NO-7 PICTURE ZZZ9.
138                       03 CSFP PICTURE A(6). JUSTIFIED RIGHT.
139                       03 NO-8 PICTURE ZZZ9.
140                       03 NO-9 PICTURE ZZZ9.
141                       03 NO-10 PICTURE ZZZ9.
142                 01 TOTS.
143                    02 FILLER PICTURE X(4).
144                    02 FILLER PICTURE X.
145                    02 FILLER-Z PICTURE X(131).
146                    02 TOT REDEFINES FILLER-Z.
147                         03 ORGAN-2 PICTURE A(6).
148                         03 FILLER-1 PICTURE ZZZ9.
149                         03 FILLER-5 PICTURE ZZZ9.
150                         03 DETAILS OCCURS 9.
151                              04 FILLER-2 PICTURE A(5) JUSTIFIED RIGHT.
152                              04 FILLER-3 PICTURE ZZZ9.
153/
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153                         04 FILLER-4 PICTURE ZZZ9.
154                 01 TITLE.
155                     02 FILLER PICTURE X(4).
156                     02 FILLER PICTURE X.
157                     02 FILLER-X PICTURE X(131).
158                     02 TITLE-0 REDEFINES FILLER-X.
159                         03 TITLE-1 PICTURE A(30).
160                         03 TOTAL PICTURE A(7) JUSTIFIED RIGHT.
161                         03 NUMBER-1 PICTURE ZZZ9.
162                         03 TITLE-2 PICTURE A(15) JUSTIFIED RIGHT.
163                         03 NUMBER-2 PICTURE ZZZ9.
164                         03 TITLE-3 PICTURE A(12) JUSTIFIED RIGHT.
165                         03 NUMBER-3 PICTURE ZZZ9.
166                         03 TITLE-4 PICTURE A(12) JUSTIFIED RIGHT.
167                         03 NUMBER-4 PICTURE ZZZ9.
168                         03 TITLE-5 PICTURE A(12) JUSTIFIED RIGHT.
169                         03 NUMBER-5 PICTURE ZZZ9.
170                         03 FILLER PICTURE X(6).
171                         03 NUMBER-6 PICTURE ZZZ9.
172                         03 FILLER PICTURE X(13).
173                 WORKING-STORAGE SECTION.
174                     77   C PICTURE S9999 COMPUTATIONAL.
175                     77   X PICTURE S9999 COMPUTATIONAL.
176                     77   Y PICTURE S9999 COMPUTATIONAL.
177                     77   Z PICTURE S9999 COMPUTATIONAL.
178                     77   COUNT PICTURE S9999 COMPUTATIONAL.
179                     77   GP PICTURE AA.
180                 01 CUMULATIVE-TOTALS.
181                     02 GRAND TOT PICTURE S9(4) COMPUTATIONAL.
182                     02 NG-TOT PICTURE S9(3) COMPUTATIONAL.
183                     02 P-CENT PICTURE S999 COMPUTATIONAL.
184                     02 GP-TOT PICTURE S999 COMPUTATIONAL.
185                     02 GP-POS-TOT PICTURE S999 COMPUTATIONAL.
186                     02 FOLLOW-2-FAIL PICTURE S999 COMPUTATIONAL.
187                     02 FOLLOW-3-FAIL PICTURE S999 COMPUTATIONAL.
188                     02 NOT-REQUESTED PICTURE S999 COMPUTATIONAL.
189                     02 FIGURE PICTURE S999 COMPUTATIONAL.
190                     02 UIB-TOT PICTURE S999 COMPUTATIONAL.
191                     02 SEROTYPE-TOT OCCURS 9 PICTURE 99.
192                     02 FILLER PICTURE XX.
193                     02 RELAPSE-P-CENT PICTURE S999 COMPUTATIONAL.
194                     02 CLIN-P-CENT PICTURE S999 COMPUTATIONAL.
195                 01 SIX-REC.
196                     02 SIX OCCURS 6 TIMES.
197                         03 ORG-TOT OCCURS 3 TIMES PICTURE S999 COMPUTATIONAL.
198                         03 NIN OCCURS 9 TIMES.
199                             04 P-COR PICTURE S999 COMPUTATIONAL.
200                             04 K-COR PICTURE S999 COMPUTATIONAL.
201                             04 T-COR PICTURE S999 COMPUTATIONAL.
202                             04 S-COR PICTURE S999 COMPUTATIONAL.
203/
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203                     04 PC OCCURS 3 TIMES PICTURE S999 COMPUTATIONAL.
204                     04 DRUG OCCURS 3 TIMES PICTURE S999 COMPUTATIONAL.
205           01 NINE-REC.
206               02 NINE OCCURS 9 TIMES.
207                   03 TREATMENT-TOT PICTURE S999 COMPUTATIONAL.
208                   03 DRUG-SUCCESS-TOT PICTURE S999 COMPUTATIONAL.
209                   03 DRUG-FAIL-TOT PICTURE S999 COMPUTATIONAL.
210                   03 RELAPSE-TOT PICTURE S999 COMPUTATIONAL.
211                   03 NO-RELAPSE PICTURE S999 COMPUTATIONAL.
212                   03 CLIN-SUC-TOT PICTURE S999 COMPUTATIONAL.
213                   03 CLIN-FAIL-TOT PICTURE S999 COMPUTATIONAL.
214           01 TWO-REC.
215               02 TWO OCCURS 2 TIMES.
216                   03 SEX-TOT PICTURE S999.
217                   03 MARRIED-TOT PICTURE S999.
218                   03 SINGLE-TOT PICTURE S999.
219                   03 AGE-TOT OCCURS 11 PICTURE S999.
220                   03 SYMP-TOT OCCURS 11 PICTURE S999.
221                   03 BIOCHEM-TOT PICTURE S999.
222                   03 AGGLUT-TOT PICTURE S999.
223                   03 SEROTOT PICTURE S999.
224           01 DITM-REC.
225               02 DITM OCCURS 600 TIMES.
226                   03 MARKER-3-WS PICTURE S999 COMPUTATIONAL.
227                   03 NG-1 PICTURE S9 COMPUTATIONAL.
228                   03 NG-2 PICTURE S9 COMPUTATIONAL.
229                   03 NG-3 PICTURE S9 COMPUTATIONAL.
230           PROCEDURE DIVISION.
231           STEP-0.
232               OPEN INPUT CARDIN-FILE, OUTPUT CARDOUT-FILE.
233               COMPUTE GP-TOT = 0, COMPUTE GP-POS-TOT = 0.
234               PERFORM MARK-1 VARYING X FROM 1 BY 1 UNTIL X = 7 AFTER C
235               FROM 1 BY 1 UNTIL C = 4.
236               PERFORM MARK-2 VARYING X FROM 1 BY 1 UNTIL X = 7 AFTER C
237               FROM 1 BY 1 UNTIL C = 10.
238               PERFORM MARK-3 VARYING X FROM 1 BY 1 UNTIL X = 7 AFTER C
239               FROM 1 BY 1 UNTIL C = 10 AFTER Z FROM 1 BY 1 UNTIL Z = 4.
240               PERFORM CALC-0 VARYING X FROM 1 BY 1 UNTIL X = 10.
241               PERFORM MARK-0 VARYING X FROM 1 BY 1 UNTIL X = 3 AFTER Y
242               FROM 1 BY 1 UNTIL Y = 12.
243               GO TO STEP-1.
244           CALC-0.
245               MOVE ZEROS TO TREATMENT-TOT (X), DRUG-SUCCESS-TOT (X),
246               DRUG-FAIL-TOT (X), RELAPSE-TOT (X), NO-RELAPSE (X),
247               CLIN-SUC-TOT (X), CLIN-FAIL-TOT (X).
248           MARK-0. MOVE ZEROS TO SEX-TOT (X), MARRIED-TOT (X),
249               AGE-TOT (X, Y), SYMP-TOT (X, Y), SINGLE-TOT (X),
250               BIOCHEM-TOT (X), AGGLUT-TOT (X), SEROTOT (X).
251           MARK-1.
252               MOVE ZEROS TO ORG-TOT (X, C).
253           MARK-2.
254/
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254             MOVE ZEROS TO P-COR (X, C), K-COR (X, C) T-COR (X, C),
255             S-COR (X, C).
256         MARK-3.
257             MOVE ZEROS TO PC (X, C, Z), DRUG (X, C, Z).
258         STEP-1.
259             COMPUTE Y = 1.
260             COMPUTE COUNT = 1.
261         STEP-2.
262             READ CARDIN-FILE AT END GO TO OUTSTEP.
263             GO TO STEP-3, STEP-4, STEP-5, STEP-6, STEP-7,
264             DEPENDING ON MARKER-1.
265         STEP-A.
266             MOVE SPACES TO FILLER-X.
267             MOVE CORRESPONDING HEADER-IN TO TITLE-O.
268             GO TO PRINT-1, PRINT-2, PRINT-3, PRINT-4, PRINT-5, PRINT-6,
269             PRINT-7.
270             DEPENDING ON MARKER-5.
271         PRINT-1.
272             MOVE GRAND-TOT TO NUMBER-1.
273             MOVE NG-TOT TO NUMBER-2.
274             WRITE TITLE AFTER ADVANCING 3 LINES.
275             PERFORM CALC-14 VARYING X FROM 1 BY 1 UNTIL X = 7 AFTER
276             C FROM 1 BY 1 UNTIL C = 10 AFTER Y FROM 1 BY 1 UNTIL Y = 4.
277             GO TO STEP-1.
278         PRINT-2.
279             MOVE GP-TOT TO NUMBER-1.
280             MOVE GP-POS-TOT TO NUMBER-2.
281             WRITE TITLE AFTER ADVANCING 3 LINES.
282             GO TO STEP-1.
283         PRINT-3.
284             MOVE GP-TOT TO NUMBER-1.
285             MOVE GRAND-TOT TO NUMBER-2.
286             MOVE FOLLOW-2-FAIL TO NUMBER-3.
287             MOVE FOLLOW-3-FAIL TO NUMBER-4.
288             MOVE UIB-TOT TO NUMBER-5.
289             WRITE TITLE AFTER ADVANCING 3 LINES.
290             GO TO STEP-1.
291         PRINT-4. WRITE TITLE AFTER ADVANCING 3 LINES.
292             GO TO STEP-1.
293         PRINT-5. MOVE SEX-TOT (Y) TO NUMBER-1. MOVE MARRIED-TOT (Y) TO
294             NUMBER-2, MOVE SINGLE-TOT (Y) TO NUMBER-3.
295             MOVE SYMP-TOT (Y, 6) TO NUMBER-4.
296             WRITE TITLE AFTER ADVANCING 3 LINES. ADD 1 TO Y, GO TO
297             STEP-2.
298         PRINT-6.
299             MOVE AGE-TOT (COUNT 1) TO NUMBER-1.
300             MOVE AGE-TOT (COUNT 2) TO NUMBER-2.
301             MOVE AGE-TOT (COUNT 3) TO NUMBER-3.
302             MOVE AGE-TOT (COUNT 4) TO NUMBER-4.
303             MOVE AGE-TOT (COUNT 5) TO NUMBER-5.
304             WRITE TITLE AFTER ADVANCING 3 LINES.
305/
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305             GO TO STEP-2.
306         PRINT-7.
307             MOVE AGE-TOT (COUNT 6) TO NUMBER-1.
308             MOVE AGE-TOT (COUNT 7) TO NUMBER-2.
309             MOVE AGE-TOT (COUNT 8) TO NUMBER-3.
310             MOVE AGE-TOT (COUNT 9) TO NUMBER-4.
311             MOVE AGE-TOT (COUNT 10) TO NUMBER-5.
312             ADD 1 to COUNT.  WRITE TITLE AFTER ADVANCING 3 LINES.
313             GO TO STEP-2.
314         CALC-14.
315             IF ORG-TOT (X, Y) = 0 COMPUTE PC (X, C, Y) = 0 ELSE COMPUTE
316             PC (X, C, Y) ROUNDED = DRUG (X, C, Y) * 100 / ORG-TOT (X, Y).
317         STEP-3.
318             IF MARKER-4 = 1 MOVE MARKER-3 TO MARKER-3-WS (COUNT).
319             MOVE NO-GRO TO NG-1 (COUNT).
320             IF MARKER-4 = 2 AND MARKER-3 = MARKER-3-WS (COUNT).
321             MOVE NO-GRO TO NG-2 (COUNT).
322             IF MARKER-4 = 3 AND MARKER-3 = MARKER-3-WS (COUNT).
323             MOVE NO-GRO TO NG-3 (COUNT), ADD 1 TO COUNT.
324             IF NO-GRO = 2 AND MARKER-4 = 2 ADD 1 TO FOLLOW-2-FAIL.
325             GO TO STEP-2.
326             IF NO-GRO = 2 AND MARKER-4 = 3 ADD 1 TO FOLLOW-3-FAIL.
327             GO TO STEP-2.
328             IF NO-GRO = 3 ADD 1 TO NOT-REQUESTED, GO TO STEP-2.
329             ADD1 TO GRAND-TOT OF CUMULATIVE-TOTALS.
330             ADD NO-GRO TO NG-TOT OF CUMULATIVE-TOTALS.
331             ADD UIB TO UIB-TOT.
332             PERFORM CALC THRU CALC-7 VARYING X FROM 1 BY 1 UNTIL
333             X = 7 AFTER C FROM 1 BY 1 UNTIL C = 10.
334             PERFORM CALC-1 VARYING X FROM 1 BY 1 UNTIL X = 7.
335             GO TO STEP-2.
336             IF MARKER-7 = 0 NEXT SENTENCE ELSE GO TO STEP-2.
337             IF ORG-TYPE (X) = 1 PERFORM COLI VARYING X FROM 1 BY 1 UNTIL
338             X = 3.
339             PERFORM COLI-1 VARYING X FROM 1 BY 1 UNTIL X = 10.
340             GO TO STEP-2.
341         COLI.  IF DISC-SEN (1) = (X - 1) ADD BIOCHEM TO BIOCHEM-TOT (X),
342             ADD AGGLUT TO AGGLUT-TOT (X).
343             IF SEROTYPE  9 ADD 1 TO SEROTOT (X).
344         COLI-1. IF SEROTYPE = X ADD 1 TO SEROTYPE-TOT (X).
345         CALC-1. ADD ORG-TYPE (X) TO ORG-TOT (X, 1).
346             IF MARKER-4 = 1 AND RECUR-MARKER = 0 ADD ORG-TYPE (X) TO
347             ORG-TOT (X, 2).
348             ELSE ADD ORG-TYPE (X) TO ORG-TOT (X, 3).
349         CALC.
350             IF ORG-TYPE (X) = 1 NEXT SENTENCE, ELSE GO TO CALC-7.
351             ADD DISC-SEN (C) TO DRUG (X, C, 1).
352             IF MARKER-4 = 1 AND RECUR-MARKER = 0 ADD DISC-SEN (C) TO
353             DRUG (X, C, 2), ELSE ADD DISC-SEN (C) TO DRUG (X, C, 3).
354             IF DISC-SEN (1) = 0 ADD DISC-SEN (C) TO P-COR (X, C).
355             IF DISC-SEN (5) = 0 ADD DISC-SEN (C) TO K-COR (X, C).
356             IF DISC-SEN (7) = 0 ADD DISC-SEN (C) TO T-COR (X, C).
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357             IF DISC-SEN (9) = 0 ADD DISC-SEN (C) TO S-COR (X, C).
358           CALC-7.  EXIT.
359           STEP-4.
360               MOVE SPACES TO FILLER-Z.
361               MOVE ORGAN-1 TO ORGAN-2.
362               PERFORM CALC-2 VARYING X FROM 1 BY 1 UNTIL X = 10.
363               IF MARKER-6 = 1 PERFORM CALC-3 VARYING X FROM 1 BY 1 UNTIL
364               X = 10.
365               IF MARKER-6 = 2 PERFORM CALC-8 VARYING X FROM 1 BY 1 UNTIL
366               X = 10.
367               IF MARKER-6 = 3 PERFORM CALC-9 VARYING X FROM 1 BY 1 UNTIL
368               X = 10.
369               IF MARKER-6 = 4 PERFORM CALC-10 VARYING X FROM 1 BY 1 UNTIL
370               X = 10.  MOVE ORG-TOT (Y, 2) TO FILLER-1.
371               MOVE ORG-TOT (Y, 3) TO FILLER-5,
372               ELSE MOVE ORG-TOT (Y, 1) TO FILLER-1.
373               ADD 1 TO Y.
374               WRITE TOTS AFTER ADVANCING 3 LINES.
375               GO TO STEP-2.
376           CALC-2.  MOVE ANTIBIOT (X) TO FILLER-2 (X).
377           CALC-3.  MOVE DRUG (Y, X, 1) TO FILLER-3 (X).
378               MOVE PC (Y, X, 1) TO FILLER-4 (X).
379           CALC-8.
380               IF ORG-TOT (Y, 1) - DRUG (Y, 1, 1) = 0 COMPUTE FIGURE = 0.
381               ELSE COMPUTE FIGURE = (ORG-TOT (Y, 1) - DRUG (Y, 1, 1) -
382               P-COR (Y, X))* 100 / (ORG-TOT (Y, 1) - DRUG (Y, 1, 1)).
383               MOVE FIGURE TO FILLER-3 (X).
384               IF ORG-TOT (Y, 1) - DRUG (Y, 5, 1) = 0 COMPUTE FIGURE = 0.
385               ELSE COMPUTE FIGURE = (ORG-TOT (Y,1) - DRUG (Y, 5, 1) -
386               K-COR (Y, X)) * 100 / (ORG-TOT (Y, 1) - DRUG (Y, 5, 1)).
387               MOVE FIGURE TO FILLER-4 (X).
388           CALC-9.
389               IF ORG-TOT (Y, 1) - DRUG (Y, 7, 1) = 0 COMPUTE FIGURE = 0.
390               ELSE COMPUTE FIGURE = (ORG-TOT (Y, 1) - DRUG (Y, 7, 1) -
391               T-COR (Y, X)) * 100 / (ORG-TOT (Y, 1) - DRUG (Y, 7, 1).
392               MOVE FIGURE TO FILLER-3 (X).
393               IF ORG-TOT (Y, 1) - DRUG (Y, 9, 1) = 0 COMPUTE FIGURE = 0.
394               ELSE COMPUTE FIGURE = (ORG-TOT (Y, 1) - DRUG (Y, 9, 1) -
395               S-COR (Y, X)) * 100 / (ORG-TOT (Y, 1) - DRUG (Y, 9, 1)).
396               MOVE FIGURE TO FILLER-4 (X).
397           CALC-10.
398               MOVE PC (Y, X, 2) TO FILLER-3 (X).
399               MOVE PC (Y, X, 3) TO FILLER-4 (X).
400           STEP-5.
401               IF MALE COMPUTE Y = 1, ELSE IF FEMALE COMPUTE Y = 2.
402               ADD 1 TO SEX-TOT (Y).
403               IF MARRIED ADD 1 TO MARRIED-TOT (Y), ELSE IF SINGLE ADD 1 TO
404               SINGLE-TOT (Y).
405               IF AGE   6 ADD 1 TO AGE-TOT (Y, 1).
406               PERFORM ZED VARYING X FROM 2 BY 1 UNTIL X = 12.
407               ADD 1 TO GP-TOT OF CUMULATIVE-TOTALS.
408               PERFORM CALC-11 THRU CALC-5 VARYING Y FROM 1 BY 1 UNTIL
409               Y = 10.  GO TO STEP-2.
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410             ZED.
411                 COMPUTE C = 10 * (X - 1) - 5. COMPUTE Z = 10 * (X - 1) + 6.
412                 IF AGE   C NEXT SENTENCE.
413                 IF AGE   Z ADD 1 TO AGE-TOT (Y, X).
414                 IF SYMPTOMS (X) = 1 ADD 1 TO SYMP-TOT (Y, X).
415             CALC-11.
416                 IF TREATMENT (Y) = 1 NEXT SENTENCE, ELSE GO TO CALC-5.
417                 ADD 1 TO TREATMENT-TOT (Y).
418                 IF CLIN-SUCCESS = 1 ADD 1 TO CLIN-SUC-TOT (Y), ELSE IF
419                 CLIN-SUCCESS = 0 ADD 1 TO CLIN-FAIL-TOT (Y).
420                 PERFORM CALC-4 THRU CALC-6 VARYING X FROM 1 BY 1 UNTIL
421                 X = 601.
422             CALC-5.  EXIT.
423             CALC-4.
424                 IF MARKER-3 = MARKER-3-WS (X) NEXT SENTENCE,
425                 ELSE GO TO CALC-6.
426                 IF NG-1 (X) = 1 GO TO CALC-6.
427                 ADD 1 TO GP-POS-TOT OF CUMULATIVE-TOTALS.
428                 IF NG-2 (X) = 1 ADD 1 TO DRUG-SUCCESS-TOT (Y).
429                 IF NG-2 (X) = 0 ADD 1 TO DRUG-FAIL-TOT (Y), GO TO CALC-6.
430                 IF NG-3 (X) = 0 ADD 1 TO RELAPSE-TOT (Y).
431                 IF NG-3 (X) = 1 ADD 1 TO NO-RELAPSE (Y).
432             CALC-6.  EXIT.
433             STEP-6.
434                 MOVE SPACES TO FILLER-Y.
435                 MOVE CORRESPONDING PATIENT-IN TO PAT-TOT.
436                 MOVE DRUG-SUCCESS-TOT (COUNT) TO NO-1.
437                 MOVE DRUG-FAIL-TOT (COUNT) TO NO-2.
438                 MOVE TREATMENT-TOT (COUNT) TO DRUG-TOT.
439                 MOVE NO-RELAPSE (COUNT) TO NO-5.
440                 MOVE RELAPSE-TOT (COUNT) TO NO-6.
441                 COMPUTE FIGURE = DRUG-SUCCESS-TOT (COUNT), +
442                 DRUG-FAIL-TOT (COUNT).
443                 IF FIGURE = 0 COMPUTE P-CENT = 0, ELSE
444                 COMPUTE P-CENT ROUNDED = DRUG-SUCCESS-TOT (COUNT) * 100 /
445                 FIGURE.
446                 MOVE P-CENT TO NO-3.
447                 COMPUTE FIGURE = RELAPSE-TOT (COUNT) + NO-RELAPSE (COUNT).
448                 IF FIGURE = 0 COMPUTE RELAPSE-P-CENT = 0,
449                 ELSE COMPUTE RELAPSE-P-CENT ROUNDED = RELAPSE-TOT (COUNT), *
450                 100 / FIGURE.
451                 MOVE RELAPSE-P-CENT TO NO-4.
452                 COMPUTE FIGURE = RELAPSE-P-CENT / 100 * P-CENT.
453                 MOVE FIGURE TO NO-7.
454                 MOVE CLIN-SUC-TOT (COUNT) TO NO-8.
455                 MOVE CLIN-FAIL-TOT (COUNT) TO NO-9.
456                 COMPUTE FIGURE = CLIN-SUC-TOT (COUNT) * CLIN-FAIL-TOT
457                 (COUNT).  IF FIGURE = 0 COMPUTE CLIN-P-CENT = 0, ELSE
458                 COMPUTE CLIN-P-CENT ROUNDED = CLIN-SUC-TOT (COUNT) * 100 /
459                 FIGURE.  MOVE CLIN-P-CENT TO NO-10.
460                 WRITE PATIENT-TOTALS AFTER ADVANCING 2 LINES.
461                 ADD 1 TO COUNT.
462/
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462             GO TO STEP-2.
463         STEP-7.  MOVE SPACES TO FILLER-Y.
464             MOVE SEROTYPE-TOT (1) TO NO-1.
465             MOVE SEROTYPE-TOT (2) TO NO-2.
466             MOVE SEROTYPE-TOT (3) TO NO-3.
467             MOVE SEROTYPE-TOT (4) TO NO-5.
468             MOVE SEROTYPE-TOT (5) TO NO-6.
469             MOVE SEROTYPE-TOT (6) TO NO-4.
470             MOVE SEROTYPE-TOT (7) TO NO-7.
471             MOVE SEROTYPE-TOT (8) TO NO-8.
472             MOVE SEROTYPE-TOT (9) TO NO-9.
473             WRITE PATIENT-TOTALS AFTER ADVANCING 3 LINES.
474             GO TO STEP-2.
475         OUTSTEP. CLOSE CARDIN-FILE, CARDOUT-FILE, STOP RUN.
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APPENDIX II

In the following pages there are copies of the various request forms and letters sent to the patients, of instructions to nurses on the taking of specimens, and of questionaires to the general practitioners and patients.

1. Copy of the form used initially in the clinical trial. In this standard form there was no provision for recording the marital state of the patient, or the treatment which was prescribed.

ABERDEEN GENERAL HOSPITALS

Request for bacteriological examination

Surname ..... Age .....

Christian names ..... Sex .....

Address ..... Under care of .....

Provisional diagnosis .....

Clinical abstract .....

Nature of specimen ..... Examination requested .....

Signiture of doctor ..... Date .....

2. The following request forms were devised for the trial. The three types differed only in the antibiotics listed after "Treatment prescribed". The first type was used initially, and types (2) and (3) for the controlled trial. In the controlled trial each stream-inoculum outfit delivered to the general practitioners contained a bottle of medicine labelled either 'The Treatment' or 'The Tablets', and subsequently revealed to contain either penicillin or sulphonamide respectively. An appropriate form was included with each outfit.

UNIVERSITY OF ABERDEEN

Department of Bacteriology

Mr/Mrs/Miss .....

Christian name .....

Age .....

Address .....

.....

Clinical features .....

.....

Treatment prescribed: (Please tick)	(1)	(2)	(3)
	None	None	None
	Penicillin G Sulphonamide	'Treatment'	'Tablets'
	Tetracycline	Tetracycline	Tetracycline
	Furadantin	Furadantin	Furadantin
	Negram	Negram	Negram
	Ampicillin	Ampicillin	Ampicillin
	Other	Other	Other .....

Date .....

Signed .....
(General practitioner)

3. The following letter was sent to patients in the clinical trial with a stream-inoculum spoon outfit, two weeks after the first specimen was received in the laboratory. The spoon was therefore inoculated by the patient about 16 - 20 days after the diagnosis had been made and treatment started.

UNIVERSITY OF ABERDEEN

Department of Bacteriology

Dear .....

Two weeks ago I received from you a urine test. The result of this has been sent to your doctor who may have put you on treatment.

Whether you have been on treatment or not, I would like you to do another test for me in the same way. I will send the result to your doctor.

I would be grateful if you would answer the two questions below with a tick.

1. Has the medicine made you better? Yes / No

2. Did you take your medicine every day? Yes / No

Any remarks:

Yours faithfully,

.....
(Dr. J. Hulbert)

4. The following letter was sent to patients in the clinical trial with a stream-inoculum outfit six weeks after the first specimen was received in the laboratory.

UNIVERSITY OF ABERDEEN

Department of Bacteriology

Dear .....

Six weeks, and four weeks ago approximately I received from you urine tests. The results of these have been sent to your doctor.

I would like you to do another test for me in the same way. The result of this test will also be sent to your doctor.

Yours faithfully,

.....
(Dr. J. Hulbert)

5. The following instructions on the collection of dip-inoculum specimens of urine were issued to nurses in the antenatal clinic of Aberdeen Maternity Hospital.

INSTRUCTIONS TO NURSES

Dip-inoculum specimens

(1) No cleansing of the perineum is required.

(2) The fundamental instruction is that the specimen must be taken without interrupting the stream of urine.

(3) Active co-operative patients may carry out the procedure themselves. Ask the patient to 'switch' the urine container into the stream of urine after about a third of the stream has been passed, and switch it out again before the end.

(4) Remove the cap of the dip-inoculum outfit. Lift the spoon out carefully, dip it into the urine, being careful to immerse the whole of the agar surface, return it to the bottle, and screw back the cap.

(5) Label and send to the bacteriology department.

6. The following instructions on the collection of a stream-inoculum specimen and a mid-stream specimen from the same patient at the same time were issued to the nurses in the antenatal clinic of Aberdeen Maternity Hospital.

INSTRUCTIONS TO NURSES

Stream-inoculum and mid-stream specimens

(1) No cleansing of the perineum is required.

(2) The fundamental instruction is that the specimen must be taken without interrupting the stream of urine.

(3) Active co-operative patients may carry out the proceedure themselves. Remove the spoon from its container and take the cap off the urine bottle. Ask the patient to 'switch' first the spoon and then the urine bottle into the stream of urine after about a third of the stream has been passed. The spoon need stay in the stream only very briefly, and only a very small quantity is required in the bottle. It is essential that both procedures be completed before the stream is finished.

(4) Replace the spoon in its container, and screw the lids onto both bottles.

(5) Label and send the specimens directly to the bacteriology laboratory, or refrigerate until this can be done.

7. The following questionaire was sent to the general practitioners who had co-operated in the clinical trial.

QUESTIONAIRE

Doctor .....

(1) The spoon method of urine culture was better than / as good as / poorer than the traditional method.

(2) The spoon method of urine culture was better than / as good as / poorer than the boric acid (urine preservative) method.

(3) Pus cell counts are/are not a very useful guide in most circumstances.

(4) Penicillin G as a first choice antibiotic in uncomplicated urinary infection was better than / as good as / poorer than the antibiotic I usually use which is .....

(5) Remarks: In particular I would welcome information about differences between penicillin and your usual antibiotic, whether favourable or unfavourable to penicillin.

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